JUL 2 1928

Number 3

hysiological Zoölogy

JULY 1928

Arthropod Hibernation. II. The Hibernation of the Ant, *ulkei* Emery - - - - - - *A. M. Holmquist* 325

of Temperature on the Development of an Insect *japonica* Newman) - - - - - *Daniel Ludwig* 358

of Error in Metabolic Gas-Exchange Measurements *G. Edward Spencer* 390

iolet Radiation and Division in *Paramecium caudatum* *Marie A. Hinrichs* 394

Physiology of the Pallet Mechanism of the Shipworm, *navalis* - - - - - - - - - *Harold F. Blum* 416

Development in Aggregates of Dissociated Cells from *Corypalma* - - - - - - - - - - *C. M. Child* 419

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS, U.S.A.
THE CAMBRIDGE UNIVERSITY PRESS, London
THE MARUZEN COMPANY, LIMITED, Tokyo
THE COMMERCIAL PRESS, LIMITED, Shanghai

PHYSIOLOGICAL ZOÖLOGY

A Quarterly Journal of Zoölogical Research

CHARLES MANNING CHILD
Managing Editor

EDITORIAL BOARD

W. C. ALLEE, *University of Chicago*
M. F. GUYER, *University of Wisconsin*
L. V. HEILBRUNN, *University of Michigan*
E. E. JUST, *Howard University*
F. R. LILLIE, *University of Chicago*
R. S. LILLIE, *University of Chicago*
W. W. SWINGLE, *University of Iowa*
C. V. TAYLOR, *Stanford University*

Physiological Zoölogy is published quarterly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. The subscription price is $6.00 a year; the price of single copies is $1.50. Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, Canary Islands, El Salvador, Argentina, Bolivia, Brazil, Colombia, Chile, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, Peru, Hayti, Uruguay, Paraguay, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Balearic Islands, and Spain. Postage is charged extra as follows: for Canada and Newfoundland, 15 cents on annual subscriptions (total $6.15), on single copies 4 cents (total $1.54); for all other countries in the Postal Union, 25 cents on annual subscriptions (total $6.25), on single copies 6 cents (total $1.56). Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4, England. Yearly subscriptions, including postage, £1. 11*s.* 3*d.* each; single copies, including postage, 7*s.* 10*d.* each.

For China: The Commercial Press, Ltd., Paoshon Road, Shanghai. Yearly subscriptions, $6.00; single copies, $1.50, or their equivalents in Chinese money. Postage extra on yearly subscriptions 25 cents, on single copies 6 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editors and manuscripts should be addressed to the Managing Editor of PHYSIOLOGICAL ZOÖLOGY, The University of Chicago, Chicago, Ill.

Entry as second-class matter applied for at the Post-office at Chicago, Illinois, under the Act of March 3, 1879.

[PRINTED IN U.S.A.]

Physiological Zoölogy

Vol. I JULY 1928 No. 3

STUDIES IN ARTHROPOD HIBERNATION. II. THE HIBERNATION OF THE ANT, FORMICA ULKEI EMERY

(Five figures)

A. M. HOLMQUIST
Zoölogical Laboratory, University of Chicago, and St. Olaf College

IN A previous publication, data were presented on the hitherto unknown life-history and habits of the mound-building ant, *Formica ulkei* Emery, which occurs in two large groups of colonies in the vicinity of Chicago, one at Palatine and the other at Palos Park, Illinois. In this region, it inhabits the open forests or forest borders. It builds mounds often 5 feet in diameter; the largest seen was 9 feet long, 7 feet wide, and 2 feet high. *F. ulkei* is common in Eastern Canada but occurs sparingly in the Transition Zone, although it is similar in appearance and habits to its near relative, *Formica exsectoides* Forel, of the eastern states.

The present report is concerned with studies upon the hibernation of this species. The work was done at the University of Chicago under the direction of Dr. W. C. Allee, to whom I am greatly indebted.

GENERAL METHODS

The study of the hibernation of *F. ulkei* was begun in the fall of 1924 and carried on through the following winter and spring. Some additional data and checking observations were obtained during the

winter of 1925–26, but the main body of the data was secured during the preceding winter. During the 1924–25 studies, the colonies at Palatine were visited regularly every 10 days between September 1 and June 26. Three of the larger nests were selected for study. All three were located along the edge of a small pond at about the same distance from the water's edge. Each nest was entered several times, each time in a different place, until the whole nest had been completely excavated. Then another nest was entered. In this way, more comparable results were obtained than if different nests had been entered on each successive trip.

On nearly every trip the nests were excavated to soil-water level. Notes were taken on the place and manner of hibernation, amount of activity shown by the ants, nest architecture, physical conditions in the nest, and other related facts. Collections of hibernating ants were made for physiological experiments on each trip. Other inhabitants of the nest were collected as observed.

Early in the fall, a large number of worker ants were collected from the field and established in large artificial nests in the laboratory, to be used as material in a series of physiological experiments. They were kept at room temperature, fed, and otherwise cared for throughout the winter.

HIBERNATION

The entrance into hibernation is gradual, involving an increasing number of individuals until the activity of the colony ceases. On September 1, the usual surface activity was noted, and a large number of worker pupae were present at the surface. However, only the upper parts of the nest were investigated on this trip. On September 10, the nest was penetrated to soil-water level (1.5 feet below the surrounding level). It was found that all the pupae had been carried to the bottom of the nest in the 6 inches of saturated soil just above the soil-water level, where they were found in groups of from two to eight. Many workers were also found in loose, small aggregations at the same level. These ants were sluggish and were evidently beginning hibernation. The number of pupae decreased on each succeeding trip, and by October 21 all pupae had emerged. The hibernating ants were found in increasing numbers in the 6-inch zone of wet clay just above the water level and were crowded

into the lower burrows in densely packed masses. Surface activity continued until November 1. The U.S. Weather Bureau records for Chicago showed a progressive decrease in mean temperature throughout November, so that it is not likely that much, if any, surface activity occurred after November 1. The retirement into hibernation, therefore, occupied a period of at least a month and a half (September 10—November 1). This retirement was taking place while other species of insects were still active, such as grasshoppers, crickets, damsel bugs (*Nabidae*), bee flies (*Bombyliidae*), *Pyrellia* (*Muscidae*), syrphus flies (*Syrphidae*), etc. Some of these insects complete their life-cycle during the fall, but the nabid bugs and *Pyrellia* are hibernators in this region.

Emergence from hibernation in spring is likewise gradual. On April 2, all the frost was out of the ground, and worker ants were again actively crawling about on the surface of all the mounds. It was not until May 14, however, that the hibernation aggregations had completely broken up. The emergence from hibernation, therefore, is as slow a process as the entry into it in fall.

The hibernation period for this species was a prolonged one (5–8 months). The winter of 1924–25 was not unusually long for the Chicago region, and it is assumed that this period is normal for this species. As compared with the hibernation period of most insects, it is a long one.

Wingless females were found on various occasions throughout the winter and spring. Many such females were taken in each nest, usually in the dense aggregations of hibernating workers at the bottom of the nest; but on February 7 several females were found in the frozen portion of a mound located on high ground; on no other occasion were they taken in the frozen surface earth. The mature females and the workers are the only castes which hibernate; no immature stages were ever found in the nests during the cold season.

Although the place of hibernation for the vast majority of the individuals in the three nests regularly visited was in the 6-inch zone just above the soil-water level, scattered individuals were found in the upper frozen portions of the mound throughout the winter. But this arrangement is not the only one. On December 13, a small nest

(only 2.5 feet in diameter) was opened. Like the other nests studied, it was located close to the edge of the pond. It was excavated to soil-water level. The mound was unfrozen on this date because of the rains of the week. In the upper portion of the mound, numerous unaggregated workers were found; while the aggregations of hibernating ants occurred in the lower part of the black, surface soil, considerably above the soil-water level. In this small nest, then, the place of hibernation was in the upper or middle levels, instead of at the water level. On February 7, a large nest was opened which was located on top of one of the low hills and removed 40–50 yards from any low spot. This nest was penetrated to a depth of 4 feet without finding any indication of soil water. A large number of ants (workers and wingless females) were found in the frozen earth at the top—some aggregated, some not. Typical aggregations of hibernating ants were found at the bottom of the excavation, however. It is obvious that the main hibernation site is not always at water level but is sufficiently removed from the surface to escape frost. The ants of the low-ground nests are necessarily limited in their retreat from the frost line, and hence are found in the zone just above the water level.

MOISTURE CONDITIONS IN THE NEST IN WINTER

The moisture factor in the environment of the ants is variable in the fall and spring. There is a considerable precipitation during these two seasons in the Chicago region. Snow alternates with rain until the next season is definitely established. In some years, the snowfall is heavy and the ground is covered with snow all winter; in other years, the ground remains bare nearly all winter.

The winter of 1924–25 was unusual in that a sleet storm during the week of December 20 left a thick covering of ice which was shortly afterward covered with snow. The maximum depth of snow recorded was 5 inches. This snow and ice remained all winter until about February 7, when the spring rains began.

The surface soil of the mound is subject to alternate freezing and thawing in spring and fall, but during the winter it remains frozen. The mound was frozen for the winter by December 13 to a depth of 1 foot. The soil of the larger mounds froze to a depth of

about 2 feet. This frost remained in the ground until February 24, when the surface soil began to thaw; but it was not until April 2 that all frost had disappeared.

The few ants that spend the winter in the upper portions of the nest, therefore, are subject to marked fluctuations in the moisture factor of their environment in fall and spring. But when frost has entered the ground, this moisture factor becomes very constant until the spring thaw. During the winter, the ants that inhabit these upper galleries are usually surrounded with frost crystals. They seem to suffer no inconvenience, however, and survive the uncertain conditions of their nest.

The moisture conditions were found to be very different at the bottom of the nest. Here the ants were surrounded with saturated clay just above the level of soil water, in the case of the nests regularly studied. There were marked fluctuations of the level of the water throughout the period of observation. (See Fig. 1.) During the fall, the soil water was near the surface because of the precipitations of that season. The water receded during the winter to a depth of over 5 feet.[1] With the spring thaw and rains the water rose again, to a point near the surface. No direct data are available on fluctuations in summer; but, judging from the drying up of the nearby pond, undoubtedly another recession of the water occurred, the beginning of which is indicated in Figure 1.

These probable summer fluctuations do not come into relation—at least, not directly—with the ants because of the fact that they inhabit only the upper levels of the nest during this season. But during the winter this fluctuation of the soil-water level constitutes a real problem for the hibernating ants. Particularly is this true of those colonies located near the low areas. In the three regularly visited nests, the ants were always found in the 6–8 inch zone above the water level. How, then, do they react to the changing water level? It was observed that the hibernation aggregations were found at deeper and deeper levels in the fall, and closer and closer to the surface on each successive trip in the spring, but always just above the

[1] The rise in level shown on November 22 was the result of a short wet period. The high point shown on January 13 represents an abnormal condition due to the fact that I entered the nest in a place formerly excavated. Very few ants were obtained in this hole.

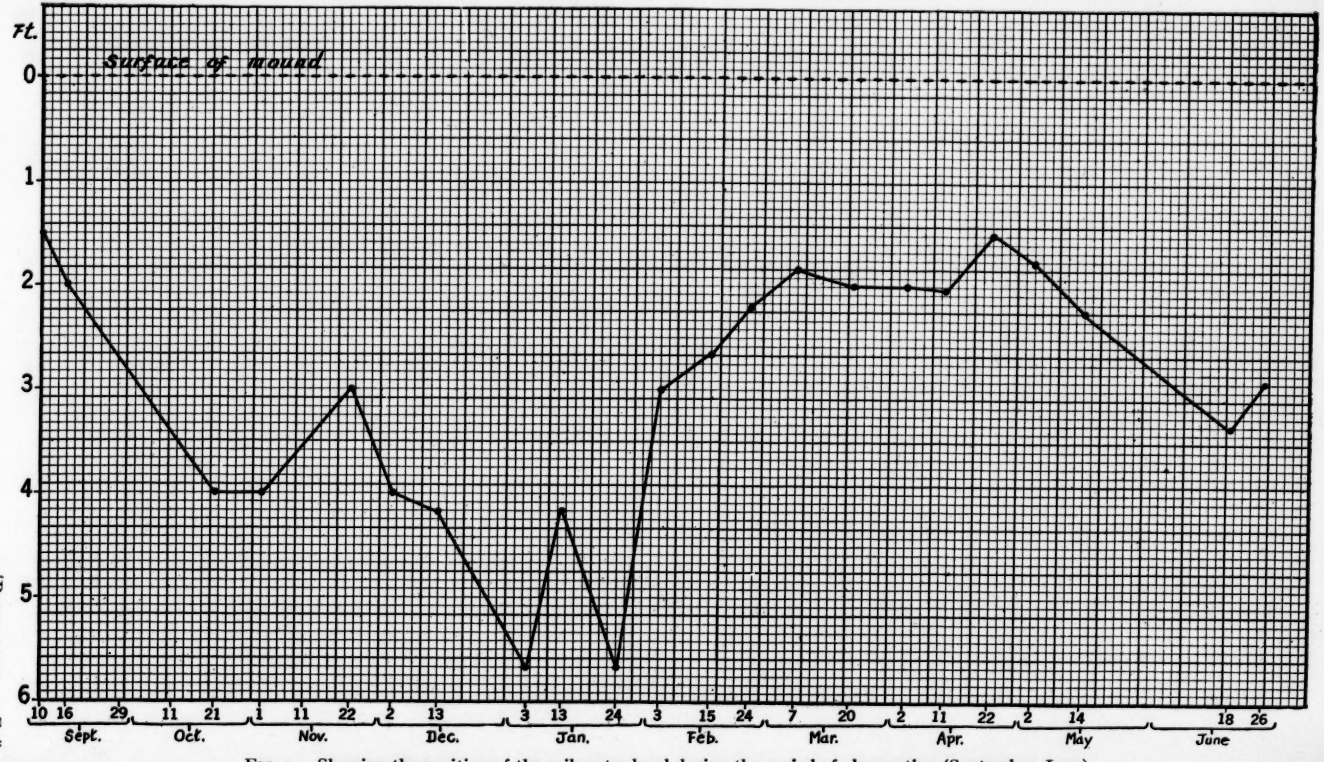


FIG. 1.—Showing the position of the soil-water level during the period of observation (September–June)

water level. These observations would seem to indicate a downward and upward migration corresponding with the fall and rise of water level in the nest. The observations were all made on colony No. 4, so that the difference in water level and position of the hibernating aggregations cannot be attributed to difference in the various nests.

Although the water level rose, the ants were not submerged as a general rule. This point was determined when, on a number of occasions, several shovelfuls of water and mud taken from below the water level were examined to see if this material contained ants. On no occasion were ants found in this submerged soil. However, on one trip (December 13), a vertical burrow was exposed about an inch above the water line. This burrow was filled with a dense mass of ants, as all the burrows are at this level. The mass was removed with a pair of forceps as far down as the forceps could reach (considerably below the water line). After all the ants had been removed, water filled the burrow to a point above the location of the ants at the lowest level. No water could be seen in the burrow before the ants were removed. It is probable that the dense mass of ants trapping bubbles of air had prevented the rise of the water in this burrow.

It is probable, therefore, that the ants of the lowest levels do come in contact with the water and may be submerged for a time before they can make their escape. But that they readjust their position I am convinced. And should they become submerged, they are undoubtedly protected by the fact that they, like other ants, can stand submergence for some time in cold water without injury. *Lasius latipes* can be submerged in cold water for 27 hours; *Camponotus pennsylvanicus*, for 70 hours; and *Pogonomyrmex barbatus* has been able to survive a flood of several days' duration.[1] This experiment was not performed with *F. ulkei;* but it is entirely probable that this species can likewise withstand submergence long enough to adjust its position to the rising water, especially in view of the reduced metabolism during hibernation.

In the nest on higher ground, the moisture situation at the surface is just like that of low-ground nests in winter. But at the lower levels it was found to be somewhat different. Soil water recedes

[1] William M. Wheeler, *Ants* (Columbia University Press, 1926), pp. 83–85.

deeper into the ground in these high-ground nests, as has already been pointed out. The hibernating aggregations are not surrounded with saturated soil. However, other observations on high-ground nests showed that the water level might rise to within 2 feet of the surface, so that these nests are not without their fluctuations; but the problem is less acute.

TEMPERATURE CONDITIONS IN THE NEST IN WINTER

A series of observations was made to determine the actual temperatures occurring in the nests during the period of hibernation. Temperatures of the soil, together with atmospheric temperatures, were read every 10 days during the period of observation. All temperatures were taken with a standard centigrade thermometer. The soil temperatures were determined at three levels in the nest: (1) at the surface, in the upper 2 inches of the soil of the mound; (2) at the soil-water level in the water; and (3) midway between these two points.

The results are shown graphically in Figure 2. Only the temperatures obtained from the first two levels mentioned above are plotted. The temperatures from the middle level were intermediate between the other two. The temperatures are plotted on the ordinates, and the dates are given on the abscissas. The air temperatures obtained in the field were found to check closely with the mean temperatures for those days obtained from the U.S. Weather Bureau station located on the University of Chicago campus. No observations were made on December 24, so the mean temperature for the day as recorded by the U.S. Weather Bureau is inserted; it shows a decided drop in temperature and indicates the cold period that occurred at this time.

In general, the graphs of all temperatures show a definite downward trend beginning in fall. The lowest surface-soil temperature was recorded in early February; but it is probable that a very low point was also reached during the cold period of late December, though no data were obtained at that time. Thereafter the temperature rose gradually at first and then very suddenly when the frost left the ground. On the other hand, the temperatures of the lower parts of the nest near the water level, where the mass of ants

hibernate, gradually decreased to a point near 0.0° C., reaching this low point in late February. At this time, the temperature of this

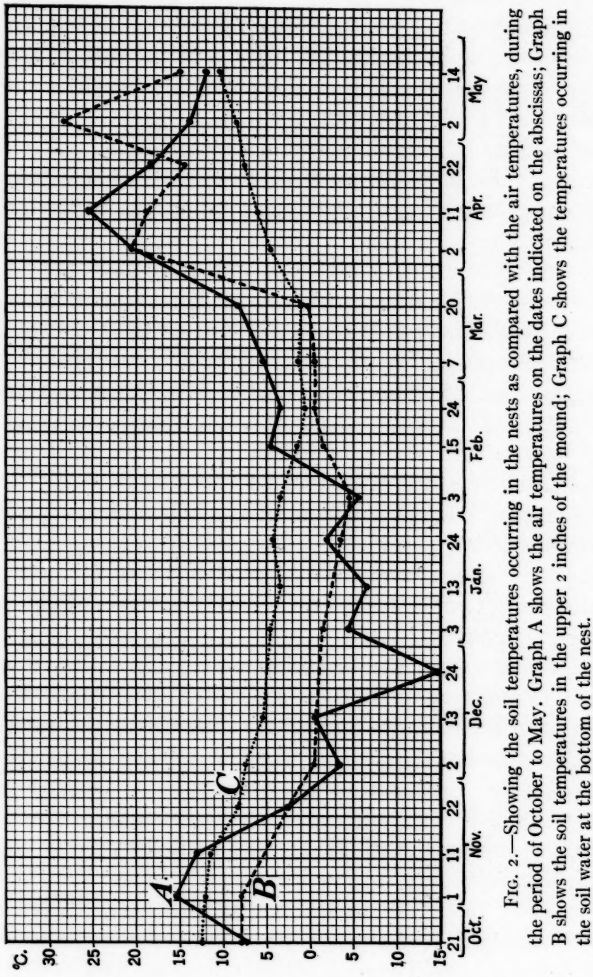


FIG. 2.—Showing the soil temperatures occurring in the nests as compared with the air temperatures, during the period of October to May. Graph A shows the air temperatures on the dates indicated on the abscissas; Graph B shows the soil temperatures in the upper 2 inches of the mound; Graph C shows the temperatures occurring in the soil water at the bottom of the nest.

level approximated that of the surface soil because the spring thaw had brought the soil water to a point near the surface. In the spring

the temperature of the soil water rose as slowly and gradually as it had fallen in the autumn.

The surface soil showed a decided fluctuation in temperature in the spring, corresponding with the fluctuations of air temperatures. The same thing undoubtedly takes place in the fall, although no complete records are available on this point. During the winter, this soil maintained a fairly constant temperature due to the insulating effect of the frost in the ground and of the covering of snow and ice. The temperatures at the water level, on the other hand, showed no fluctuations at any time, a fact of great importance to the hibernating ants.

Not only was there a difference in amount of fluctuation in temperature between the surface soil and the bottom level of the nest, but there was a difference in the actual temperatures recorded. The winter temperature of the soil water never descended below 3.5° C. until the spring thaw raised the water to 1 foot 10 inches from the surface. The soil water then assumed the temperature of that level, and the temperature descended to nearly zero in consequence. The surface-soil temperatures were found to descend to − 4.5° C., and were consistently lower than those of the soil-water level until the frost was out of the ground.

The small nest excavated on December 13 showed almost exactly the same temperatures at the various levels as the larger, regularly studied nests.

It is evident from the foregoing observations that the great masses of hibernating ants at the soil-water level benefit not only by the relative constancy of temperature but also by the higher temperature here than at the surface. The few scattered ants that spend the winter in the frozen surface soil are exposed to fluctuating temperatures in fall and spring, with danger of alternate freezing and thawing, but during the winter are surrounded by fairly constant temperatures, especially if snow remains on the ground all winter. They also have to endure a consistently lower temperature during the winter months.

RELATION TO OTHER ANIMALS IN WINTER

Like other species of ants, *Formica ulkei* shares the nest with a number of other species of animals. A large number of specimens

were collected and identified during these observations. The complete list of species has been published elsewhere.[1] These represented all degrees of relationship from symbiotic to hostile. Of the true myrmecophiles, three species of pselaphid beetles (*Batrisodes globosus* Lec., *B. spretus* Lec., and *B. schaumi* Aube), one species of staphylinid beetle (*Megastilicus formicarius* Casey), and a syrphid larva (*Microdon* sp.) were found. A carabid beetle (*Tachys incurvus* Say) of somewhat doubtful relationship was taken on several occasions during the winter as well as in the summer.

With cessation of activities in the fall, a large number of animals invade the nest and make use of the temporarily abandoned burrows for over-wintering. Among such species are *Lachnosterna* larvae and adults, land isopods, earthworms, spiders, centipedes and millipedes, wood roaches, ground beetles, and even ichneumonflies. To all these co-inhabitants, the ants are totally indifferent in winter. On no occasion were such species found among the hibernation aggregations of ants, but always in the deserted burrows.

ACTIVITY DURING THE WINTER

The degree of activity was observed throughout the hibernation period. The aggregated ants were sluggish from the beginning of hibernation and became slightly more so as the winter progressed. During the entire winter, however, the aggregated ants at the bottom maintained enough activity to be able to stagger to cover very slowly when disturbed. Those found in the surface soil remained slightly more active until frost entered the ground. Thereafter, these ants became very dormant; on some of the colder days in winter they were utterly unable to crawl and could only make feeble leg movements. The difference in degree of activity shown by the bottom and surface ants is the result of exposure to the different temperatures already noted. The bottom ants were exposed to a temperature which did not go below 3.5° C. in winter; it was only in spring, when the soil-water level rose to a point near the cold surface soil, that the temperature went lower than this. On the other hand, the surface ants are exposed to temperatures at or below zero.

The fact that the ants are able to respond to the stimuli result-

[1] *Ecology*, IX (January, 1928), pp. 83–84.

ing from exposure by crawling to cover makes it possible for them to respond to the stimuli of the encroaching water by migrating toward the surface, as already pointed out. But, although sufficient metabolism is maintained to permit such response to these strong stimuli, it is not enough to allow feeding, reproduction, care of the nest, or any other of the usual activities of summer. Any responses that are shown are emergency responses to very strong stimuli.

It is significant to note that ants brought from the field at any time during the hibernation period resumed apparently normal activity in the laboratory at room temperatures. Ants collected in the field in early fall and kept in the laboratory continued to show the usual activities all winter (feeding, nest repair and care, reproduction, and active crawling about the nest).

The degree of activity seen in *F. ulkei* during the winter is in marked contrast with that of *Camponotus herculeanus pennsylvanicus*. The latter species hibernates under the bark of logs. It is deeply lethargic during the winter months and does not readily come out of hibernation when warmed. It is much more nearly a "true" hibernator than *F. ulkei*.

WATER CONTENT OF WORKERS DURING THE WINTER

Numerous investigators (Bodine, 1921; Tower, 1906; Breitenbecher, 1918; and others) have called attention to the decrease in water content of various hibernating insects. This suggested the first line of attack on the physiology of hibernation in *F. ulkei*.

Workers were collected on each of the regular field trips, and water content determinations were made during the entire hibernation period. Three series were studied: A, workers taken at or near the surface of the nest; B, hibernating workers from the dense aggregations at the bottom of the nest; and C, workers from the large stock nests kept in the laboratory all winter at room temperature. The A series consisted of ants that were active during fall and spring but dormant while frost was in the ground during the winter; the B series were partially dormant during the entire period of observation; and the C series were active at all times. About twenty-five workers were collected on each trip from each of the levels mentioned and were brought to the laboratory in stoppered

vials or bottles containing some moist earth or leaves so as to prevent desiccating effects of a dry atmosphere. On the same day of collection, each ant was placed in a weighing bottle and weighed alive on a sensitive laboratory balance. While the ants were being handled and weighed, they were kept cool by being placed in an open window. After being weighed, all ants were killed with chloroform and placed in their respective weighing-bottles with the ground stoppers removed, and the entire series of weighing-bottles and their contents placed in a large desiccating jar. This jar contained phosphorus pentoxide as the desiccating agent, and a partial vacuum was established. The ants were then left to dry for 3 or 4 days, when they were again weighed from day to day until they came to a constant weight (7–10 days). The precentage of water content was then calculated for each specimen.

In the early weighings, it was found that the body weights varied from 2.65 to 15.9 mg. Since other investigators had observed that water content varied with weight, it was thought advisable to make the determinations only on ants in the upper limit of variation, i.e., from 7 to 12 mg. This selection was made throughout the data presented here. Adults only were used in these water-content studies; no further selection as to age was made.

The results are shown graphically in Figure 3. The points on the graphs represent the average water content of the 7–25 workers used on each date. The water content of the hibernating B series (see Graph B in the figure), though fluctuating somewhat, remained approximately at the same level during the entire period of observation; it was usually lower than that of either of the other two groups. The curve representing water content of the surface ants (see Graph A) shows a different configuration. These ants suffered a marked drop in water content from the normal of summer to the same level as that of the hibernating (B series) ants during the winter, when they, too, became dormant; but upon resuming activity in the spring, their water content again rose to normal. Graph C represents the water content of the laboratory animals (C series) which were kept active all winter at room temperature. Even in these animals, the water content decreased in the fall, but the normal amount was resumed again in January; usually these ants had a higher water content

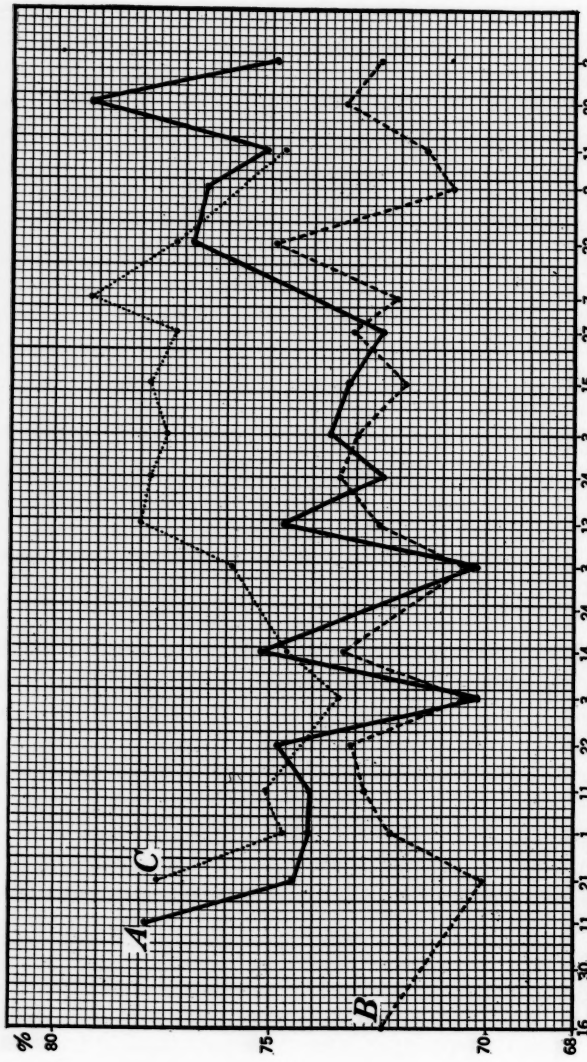


FIG. 3.—Showing percentage of water content in workers of *Formica ulkei* taken from the surface level of the nest (Graph A), from the bottom levels (Graph B), and from the laboratory (Graph C). The last mentioned were active ants; the bottom ants were hibernating; and the surface ants were active in fall and spring and dormant in winter.

than that found in freshly collected hibernating individuals. In the spring and fall the water content of the surface and laboratory animals approximated each other, as would be expected, since both were active at this time. The curve of the water content of the laboratory animals is much more free from fluctuations than that of either of the two series of field animals. This is probably to be accounted for by the lesser amount of disturbance and agitation of these animals. The hibernating animals were disturbed in collecting them in the field, in carrying them from field to laboratory, and again in making the initial weighings. This disturbance was unavoidable and was approximately the same for each collection. The values of the water content may not give the water content of the hibernating ants in nature, yet they do show a reduction of water content during hibernation.

Some checks of the above data were made the following year. To determine the normal water content of active workers in summer, a large number were collected on July 23 at Palos Park. These ants were kept in stoppered bottles containing moist (not wet) leaves and food, and on the following day were weighed in groups of from 22 to 26, instead of individually as in the work just described. They were then desiccated as before. The water content of these animals (420 in number) varied from 74.11 to 78.72 per cent, with an averabe of 75.73 per cent—approximately the same values as for the active laboratory and surface animals the year preceding. The results are given below:

No. of Animals	Percentage of Water	No. of Animals	Percentage of Water
22	76.70	25	74.98
24	78.72	25	75.22
25	76.07	25	74.96
25	74.43	23	74.62
25	65.29	25	74.91
25	76.21	25	76.29
25	75.65	25	76.33
26	75.90	25	74.11
25	76.06		
		420 total	75.73 average

On July 14, 62 workers were collected from the field and similar determinations were made. These gave the following results:

20 workers..... 74.16 per cent
22 workers..... 73.46 per cent
20 workers..... 74.63 per cent

The normal water content of active workers, though somewhat variable, is about 75 per cent.

A check on the water content during the hibernation period was also made. On March 15, a large number of hibernating workers were collected in vials each containing a quantity of moist earth and stoppered. These vials were put into a thermos bottle and surrounded with cold water from the nearby pond. A piece of cotton was packed in the top of the thermos bottle to prevent jarring of the vials within. Not all the vials could be accommodated in the thermos bottle, so the rest were packed in cotton which had been soaked in the same cold pond water and the whole was wrapped in several thicknesses of newspaper. At the laboratory, the thermos bottle and paper package containing the collecting vials were placed in a cold chamber without opening them. The following day, the ants were allowed to crawl on filter paper in the cold chamber to absorb the little droplets of moisture that had condensed on the feet and bodies of some of them. They were then placed in weighing bottles, 26–59 in each bottle, and the determinations made as before. The following are the results obtained:

No. of Animals	Percentage of Water	No. of Animals	Percentage of Water
27.....	64.92	45.....	60.77
33.....	63.80	51.....	59.92
47.....	60.72	56.....	61.94
26.....	60.25	50.....	60.78
35.....	60.33	42.....	60.11
30.....	62.11		
		442 total.....	61.26 average

These results bear out the statement made above that the absolute values for the water content are not shown in Figure 3 and that the water content descends lower than indicated.

Another series of determinations were made to see what effect prolonged low temperature, extending beyond the normal hibernation period, would have upon the water content. The animals used for this experiment were collected in April, while still hibernating.

They were kept in a refrigerator room in a large crystallizing-dish containing moist sand. The temperature of this low-temperature chamber was at or near 0.0° C. most of the time, but occasionally the temperature rose because of lack of reliable temperature control. These ants, therefore, were subjected to low temperature and occasional warmings until the time of the experiment—July 16. They were weighed in the refrigerator so as to prevent undue activity at warm temperature. The results follow:

No. of Animals	Percentage of Water
20	69.82
18	70.86
20	68.07
20	71.22
78 total	69.97 average

The water content is considerably higher in these animals than in the hibernating workers, but considerably lower than in the active ants in summer. Some water content adjustments had taken place even under these circumstances.

RESPIRATION

A profound modification of respiration takes place in winter in *F. ulkei*, as in all hibernating animals for which data are available. This fact was established in a series of studies on hibernating and non-hibernating workers.

The apparatus adopted for this part of the investigation was a respirometer devised by Crocker and Harrington. It was made primarily for use with plant materials, such as seeds, and to the writer's knowledge, has never been used before for determining the respiratory exchanges of animals. The selection of this apparatus was made because of its compactness and adaptability for use in small, cold chambers. It also has the advantage of giving data on both the oxygen consumption and the carbon-dioxide production, so that the respiratory quotient can be readily determined. By proper manipulation, it is possible to determine the amount of oxygen consumption at regular intervals for a considerable period of time. The apparatus is not sensitive to small differences in gaseous exchange and requires several hours to register changes. A 24-hour period is usually re-

quired, especially for use with low temperatures. For room temperatures it would be possible to get results in a shorter period, but it is always a matter of hours. (For a detailed description of the apparatus, see Harrington and Crocker, 1923.)

The container of the experimental material had to be modified for use with animal material. The modified container, or animal chamber, was constructed of Pyrex glass tubing 2.3 cm. in diameter and 3 cm. long, with a false bottom put in 1 cm. from the lower edge of the tubing. This bottom was perforated with holes large enough to allow the carbon dioxide produced by the ants to diffuse out of the animal chamber, but small enough to prevent the escape of the ants through them. A cover was made to fit over the top. A pair of glass knobs on each side—one on the lid, and the other on the chamber proper—permitted fastening the lid to the chamber by a wire. A small glass loop on top of the lid was convenient for raising or lowering the animal chamber in the respirometer.

Briefly, the operation of the apparatus is as follows: The volume of the respirometer is determined at the beginning of the experiment, deducting for the volume of the ants used. The apparatus is then sealed with mercury and a grease that does not readily absorb oxygen. At the close of the experiment several hours later, the volume is again determined. Potassium hydroxide is then introduced, which absorbs the accumulated carbon dioxide. This causes a change in the level of the manometer, and the volume is again calculated. The difference in the last two volumes gives the volume of the carbon dioxide produced, while the difference in the first and last volumes gives the volume of the oxygen consumed. All volumes are calculated by means of formulas worked out by the originators of the apparatus.

Two series of experiments were performed. One was done at room temperature (20°–25° C.) with worker ants kept active in the laboratory throughout the winter. The other series of experiments was done at low temperatures (3°–6° C.) on workers kept in constant temperature chambers at 3°–6° C. throughout the winter (this is the temperature to which they are exposed in nature during this period); hibernation conditions were simulated; and the ants kept in a state of hibernation. One hundred ants were used in most of

the experiments, and in most cases for a period of 24 hours. None of the experimental ants were ever used in more than one experiment. All ants were maintained in the laboratory in large stock nests amply provided with moist earth and cared for so as to keep the ants in as normal a condition as possible.

Two serious difficulties arose. In putting the ants into the animal chamber, they became unmanageable and persisted in crawling out. They were thereby greatly disturbed. Also, because of the agitation produced by handling the ants, formic acid in small drops was often excreted, the fumes of which were so strong that they killed some of the experimental animals. Consequently, it was necessary to reduce their activity while they were being handled. This was done by cooling them at a temperature a little above 0.0° C. for about an hour. The animal chamber and contained ants were then exposed to the temperature at which the experiment was to be done in the laboratory for about 3 hours, to allow the animals to recover from the low temperature to which they had just been exposed. The animal chamber with contained ants was carefully handled to reduce jarring to a minimum.

The results of twelve experiments are given in Table I. The oxygen consumption and the carbon dioxide output are shown for high and low temperatures. Each figure represents the actual gaseous exchange of 100 workers for a period of 24 hours. The respiratory quotients are given for each experiment.

The respiratory exchanges of the workers kept active all winter probably represent the normal, or very close to the normal, for the species, even though the determinations were made during the hibernation period, for these ants were actively crawling around all winter, ate food put before them, and even reproduced. These ants were quieted very easily by the cooling procedure and could therefore be handled with a minimum of disturbance during the experiment. The results, therefore, are fairly uniform. The amount of carbon dioxide produced by the 100 workers for the 24-hour period (average, 3.176 cc.) was consistently lower than the amount of oxygen consumed (average, 3.6 cc.), so that the respiratory quotient for these ants was below unity (0.81–0.95) in all the experiments of this series.

The results obtained on the hibernating workers proved to be quite different. The somewhat irregular results can be accounted for probably by the fact that cooling these ants to zero when they had previously been kept at low temperature had little effect upon them and because they were consequently agitated by handling. However, it may be pointed out that a marked reduction in respiratory exchanges was evident in these hibernating animals as compared with the active ones. Their respiratory activity was reduced to about one-third the normal amount. This reduction is not nearly

TABLE I

THE RESPIRATORY EXCHANGES OF 100 WORKERS FOR A PERIOD OF 24 HOURS AT HIGH AND LOW TEMPERATURES

DATE	ACTIVE ANTS AT 20°–25° C.			HIBERNATING ANTS AT 3°–6° C.		
	CO_2 (Cc.)	O_2 (Cc.)	Resp. Quot.	CO_2 (Cc.)	O_2 (Cc.)	Resp. Quot.
Nov. 28	1.917	2.02	0.949	.....	.....	.....
Dec. 16	3.948	4.715	0.837	.....	.....	.....
Jan. 5	3.292	3.552	0.927	0.588	0.647	0.909
Jan. 8	3.73	4.385	0.851	1.595	1.535	1.039
Jan. 19	2.46	3.02	0.815	0.743	0.950	0.782
Jan. 27	3.585	3.776	0.949	1.03	0.84	1.226
Feb. 17	.....	.....	.....	1.172	1.24	0.946
Feb. 25	.....	.....	.....	1.108	0.713	1.554
Average	3.176	3.603	0.888	1.039	0.987	1.076

as great as that reported for other hibernators, however. The carbon-dioxide production (average, 1.039 cc.) for the 100 ants during the 24-hour period was in most cases greater than the oxygen consumed (average, 0.987 cc.). The respiratory quotient was, therefore, on the average, over unity (1.076). Such a respiratory quotient has been obtained for the bee by Miss Parhon (1909), but it is in marked contrast with the respiratory quotient of hibernating mammals, in which it may descend as low as 0.3.[1]

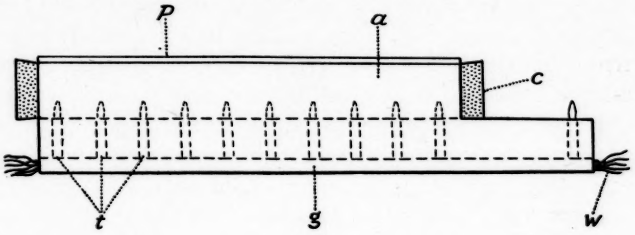
These results are imperfect and need extension, but the author has no facilities at hand for immediate prosecution of the problem.

TEMPERATURE OF ANT AGGREGATIONS

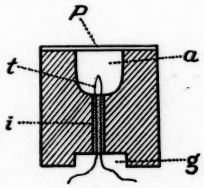
Attention has already been called to the habit of gathering in dense masses of closely packed individuals during the winter for

[1] J. Athanasiu, "Hibernation," *Dictionaire de Physiologie*, VIII (1909), 563–623.

purposes of hibernation. It seems quite natural to inquire as to the benefit, or benefits, derived from such aggregations in ants. The first thought that occurs in this connection is that of temperature conservation, or possible production, as reported for bees in winter (Philips and Demuth, 1914; also Gates, 1914).



I



II

FIG. 4.—Showing apparatus used in obtaining temperatures of ant aggregations. *I*, side view. *II*, transverse section. *a*, groove containing ants; *c*, cork to prevent escape of ants; *g*, groove to accommodate thermocouple wires; *i*, procelain insulator; *p*, glass plate covering groove; *t*, thermocouple junction; *w*, wire leads from thermocouples.

To that end a series of thermocouple experiments was begun. An apparatus was constructed of a block of maple wood 10 inches long and $1\frac{1}{2}$ inches in each of the other two dimensions. It was cut into the shape indicated in Figure 4. In the upper side of the block a groove was made with rounded bottom $\frac{3}{8}$ inch wide. This was similar in dimension to the burrows found in ant nests. Ten constantan-copper thermocouples, previously calibrated, were closely fit-

ted into holes in the floor of the groove in such a way that the junctions protruded into the groove. The junctions were ½ inch apart, so that no matter where the ants aggregated in the groove, they would always come in contact with at least one junction. One junction, however, was placed at one end of the apparatus outside of the groove, for the purpose of measuring the temperature of the air outside of the apparatus. This junction was unprotected from air currents, from heat from the experimenter's body, and from his breath; and the results obtained from it were, in consequence, inconstant. A strip of glass was glued over the groove so as to permit observations of the ants. A cork at each end of the groove closed it completely, preventing the escape of the ants. A narrow strip of filter paper was placed in the bottom of the groove and moistened to keep the ants from drying during the experiment. Readings were taken on a portable field millivoltmeter.

Ants were then put into the apparatus. These had to be cooled before handling in order to prevent excessive excretion of formic acid and consequent death to the ants, and also to facilitate their handling generally. The apparatus was then clamped to a heavy plank so as to prevent jarring of the apparatus and disturbance of the aggregation during the experiment. The millivoltmeter and the thermos bottle containing the cold junctions were placed under the shelter of a wooden box (2 feet in all three dimensions), open on one side, while the apparatus containing the ants was outside of this box. This arrangement protected the apparatus from temperature changes caused by the presence of the experimenter. It was found that the position of the aggregation could be controlled by putting an opaque cover over the spot where aggregation was desired. In this way, it was thought that, by causing an aggregation at one end, a graduated series of temperatures could be obtained from one end of the apparatus to the other if any marked difference in temperature was produced by the aggregation.

Seven experiments were performed. Some of these were run for 24 hours; others for shorter periods. One of the 24-hour experiments is representative of the rest, and the result of this one is herewith given. This experiment was performed April 15–16, 1925. One hundred twenty workers were used. Readings were taken every 2 hours

for the 24-hour period on junctions located as follows: three in the densely packed aggregation at one end, giving the temperature of a dense aggregation; one at the edge of the aggregation, giving the temperature in a loose part of an aggregation; two outside of the aggregation, not in contact with any ants, and giving the temperature of the air inside of the apparatus; and one outside of the apparatus, giving the temperature of the air. Each set of readings took 15 minutes. The experiment was done outside of the laboratory at outdoor temperatures (3°–14° C.).

The temperatures given by two of the junctions are plotted in the graphs in Figure 5. These two sets of temperatures are those of a dense aggregation, and those of the air outside of the aggregation but within the apparatus. The temperature of the air outside of the apparatus is not given because it was found that the couple was so sensitive that it indicated fluctuations due to air currents or to heat from the experimenter's body when the readings were taken. Instead, the air temperatures inside the apparatus were used for comparison because this couple was protected from such minor changes of temperature and gave a representation of the temperature as it might be conceived to be in the air of the empty parts of the burrows. Temperatures in the loose part of the aggregation were found to be intermediate between air and aggregation temperatures.

Examination of the graphs reveals a wide range of air temperatures between day and night (3.2°–14.4° C.), which afforded a fortunate opportunity for testing the temperature of the aggregation at various temperatures. It is seen that the temperatures within the aggregation follow those of the air very closely, the widest difference being only about 0.6° C. at the highest and lowest points of the graph. No striking gradations of temperature were obtained. There was neither a marked production of heat nor even a significant conservation of heat, such as is reported for the bee clusters in winter. Only a slight lag in temperature of the aggregation is indicated. All the other experiments substantiated the foregoing results. It has been suggested that this lag is not more than might be expected of a heap of dead ants closely packed together, or of other dead material. The substitution of dead ants or other dead material for the live, hibernating ants was not made in this experiment, but thermocouple

tests made by the author on the temperatures existing in various types of hibernacula (under bark, in wood, under leaves, etc.) showed that these dead materials exhibit a more or less marked lag in

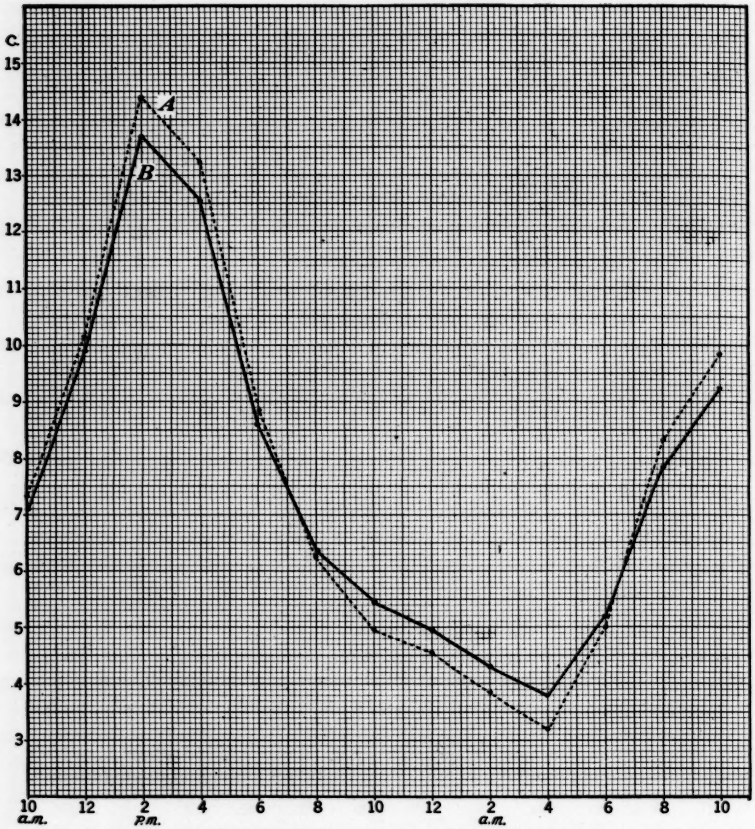


FIG. 5.—Showing the relative temperatures of the ant aggregation as compared with those of the air during a 24-hour period. Graph A gives the air temperatures observed, and Graph B the temperatures occurring in the aggregation.

temperature, even in the loosely heaped masses of dead leaves. At all events, it is evident that if there is any heat production in these aggregations of partially dormant ants, it is negligible.

DISCUSSION

A great variation in degree of dormancy may be observed among hibernating animals during the winter months. Some sink into a deep state of lethargy from which it is difficult, if not impossible, to arouse them to activity, or to bring about resumption of development in the case of the immature stages. These seem to show a periodicity in the hibernation-phenomena which is independent of temperature. This type of dormancy has been called "diapause," and species exhibiting this diapause have been called "heterodynamic" species (Roubaud, 1922). Other species are deeply dormant only during very cold weather and are easily roused from this dormancy by warming. In fact, their activity is directly related to the environmental temperature. Such species have been designated "homodynamic." Such species differences have been observed among arthropods by the author. As a result of these differences, many definitions of hibernation have been proposed. Some insist that the term "hibernation" should only be applied to those species that show the above-mentioned deep and periodic lethargy. Others include also the varying dormancy of those species which seek shelter during the cold winter months but whose activity is determined by the temperature. However one defines the term, the distinction between the deeply dormant, periodic type of hibernation and the mere numbness and inactivity caused directly by cold is a very real one and should be kept in mind. The former type has been designated "true hibernation" and the latter "false hibernation."

What type of hibernation does *F. ulkei* exhibit, and what is the nature of its hibernation?

As already pointed out, its activity is much reduced during the hibernation period but varies with the surrounding temperature. The ants readily respond to warming. Ants kept at room temperature all winter remain active, feed, reproduce, and are apparently in possession of normal activity throughout the hibernation period. They retire to winter quarters gradually, maintaining some activity at the surface until late fall. Emergence from hibernation is likewise gradual. As soon as frost is out of the ground and the soil begins to warm, surface activity begins. Nor do the immature stages require a period of exposure to low temperature for the completion

of development, as is the case of species showing diapause phenomena, for the eggs hatch in the laboratory nests at constant warm temperatures.

In view of these facts, it cannot be said that *F. ulkei* exhibits the periodic, deeply lethargic type of hibernation so common among other Hymenoptera. On the other hand, its hibernation is less profound, its activity being subject to temperature variations; in general, this species resembles roaches, *Drosophila*, *Musca domestica*, myriapods, etc., in its hibernation. If we adopt the terminology "true" and "false" hibernation, only the latter term can rightly describe the hibernation of *F. ulkei*. Or, to use Roubaud's terminology, we can refer to *F. ulkei* as being a "homodynamic" species.

F. ulkei, like other ants, is at all times very sensitive to temperature differences. In summer, its activities are confined to the parts of the nest showing the more optimum temperatures; and on very warm days, there is a careful avoidance of the hot, sun-baked portions of the mound. The larvae and pupae are shifted from one part of the mound to another in conformity with temperature and moisture conditions. In fall, when temperatures are falling, the pupae are carried to the bottom of the nest where warmer temperatures obtain. This sensitivity to temperature differences was shown by the following incident, observed in late fall. Twenty-five to thirty workers had been placed in a large, wide-mouthed bottle which I held in my hand. After a time it was observed that this entire group had gathered in a mass directly over the small area warmed by my thumb. Other incidents of a similar character have substantiated this keen sensitivity to temperature differences.

This sensitivity to temperature and the close relation of activity to temperature in this species suggest very strongly a possible explanation for retirement to hibernation quarters, as well as for the described aggregation phenomenon seen in this species. The U.S. Weather Bureau reports for the Chicago region for 1924 showed an atmospheric temperature in late August that was above normal for that season and locality. Early September was cool—in fact, 15° F. below normal. This rather abrupt lowering of air temperatures caused a similar change in the surface soil, but the deeper parts of the nest were only slightly affected. The result was a reversal of

the temperature conditions in the nest, with lowest temperatures at the surface and progressively higher temperatures toward the soil-water level. In order to escape the low temperatures in the upper parts of the nest, the ants retired as far as possible from the colder surface soil to the warmer levels below, carrying their pupae with them. The weather report for the rest of September showed a continued, but irregular, drop in temperature, causing the retirement of more and more ants to the deeper parts of the nest. This concentration of the large number of individuals of the colony in the warmest parts of the nest at the bottom results in the formation of aggregations as described. The arrival of others from above and the upward migration from below to escape the rising water level result in the crowding of the ants into the densely packed masses so commonly seen during the winter. Since it has been shown in this paper that there is little or no heat production or conservation in the aggregations, it is probable that the presence of a common advantageous site for escape from cold is in part responsible for the formation of the winter aggregations, but instinctive reactions may also play a part.

That temperature is the main factor in causing hibernation in ants has been indicated by the work of Cobelli (1903). This investigator made observations on several species of ants and found that the time of entrance into hibernation and emergence from hibernation was directly correlated with the sensitivity of these species to cold. Other investigators (McCook, 1877; Tanquary, 1913) have noted the relation between temperature and nest activity during fall and winter in other species.

Emergence in spring seems to be a temperature phenomenon also. This is shown by the fact that surface activity begins as soon as frost is out of the ground and gradually increases in intensity until all ants have left their hibernation quarters at the lower levels of the nest and become active at the surface. A possible explanation of this gradual awakening was suggested by an observation during an experiment on aggregations. The experiment was done at a temperature of 22°–25° C., so that the ants were not torpid. They were congregated at one end of the apparatus under the opaque cover. In order to move the aggregation to the opposite end of the

apparatus, this cover was shifted to the opposite end, thus exposing the aggregation to the sunlight. At once, some of the ants at the free end of the group commenced to bestir themselves, and soon they began to carry the other aggregated and inactive ants, one by one, a short distance from the rest of the heap. Some of these removed ants then engaged in the business of removing other ants; while others retired to the new darkened end of the apparatus and there began to form another aggregation. At once the condition in the nest suggested itself. The scattered ants that over-winter in the frozen soil of the mound are roused to activity in the spring when the surface soil becomes warmed. In some nests, at least, the hibernating masses of ants are forced upward at about the same time by the encroaching soil water, making them available to the ants that are already active. In view of the above-described observation, it is entirely possible that the ants of the hibernating aggregation are pulled out from the pile bodily, carried toward the warmer surface soil, and roused to activity by these stray surface ants. At least, the behavior mechanism is at hand. It is my opinion that the work of arousing the rest of the colony constitutes one of the chief activities of these surface workers during early spring. The upward migrations that take place in some of the nests because of the rising water may also bring some individuals so close to the surface that they are influenced by the warm surface soil and are thereby roused to activity.

The ants are surrounded with rather constant conditions during their hibernation. Retirement to the deeper parts of the nest below the frost line insures a non-fluctuating, slowly rising, and slowly falling temperature that remains above 0.0° C., and thereby furnishes one protection against freezing. This is in accord with the observations of Steiner (1924), who found that, although *Formica rufa* maintained a constant nest temperature of 23°–29° C. during the summer, it was unable to do so during the winter.

The ants may or may not be surrounded with saturated soil, depending upon whether the nest is located on high or low ground. When they are in saturated soil, they are so situated only incidentally in their escape from the cold temperatures of the surface. The fluctuating level of the water constitutes the most disturbing factor

in the winter environment of the colonies located on low ground. This same disturbance may enter into the environment of the high-ground colonies, but probably to a much less extent.

The reduction of physiological processes noted in this species is the usual accompaniment of hibernation. But certain differences may be pointed out.

The respiratory exchanges are not reduced nearly as much in *F. ulkei* as is reported for other hibernators. In the hedgehog, respiration is reduced 10 times (Pembrey, 1903), in the marmot 30 times (Regnault and Reiset, 1849), in the spermophile 46 times (Horvath, 1880), and in the grasshopper 8–10 times (Bodine, 1923). In comparison with these, respiration in *F. ulkei* was found to be reduced only to one-third its normal amount. Perhaps this relatively small reduction is correlated with the maintenance of a considerably greater amount of activity on the part of the ants.

The respiratory quotient is variable in the different species of hibernators during the hibernation period. In mammals, the respiratory quotient is reduced from unity to 0.6 and may be reduced to 0.3; in the frog, the respiratory quotient is increased, approaching or even exceeding unity (Athanasiu, 1909); while in the bee, the quotient is reported to remain around unity throughout the year (Parhon, 1909). The quotient in the case of *F. ulkei*, though somewhat irregular at low temperatures (0.782–1.554), hovers around unity (1.076 average). The meaning of such differences in hibernating species is not clear. Krogh (1915) has correlated respiratory quotients with the type of metabolism. According to his interpretation, if any significance attaches to the quotients determined for *F. ulkei*, it is that carbohydrate metabolism is indicated and that stored carbohydrates furnish the nourishment during the winter. But more experiments are needed on this point.

The loss in water content noted in *F. ulkei* during the winter is one of the universal physiological accompaniments of hibernation. It is a gradual process beginning in fall at the onset of cold weather and inactivity and returning to normal on the resumption of activity again. However, the reduction in water content is all the more remarkable in the case of *F. ulkei* because it occurs in spite of the fact that the animals are surrounded with saturated soil during this

period. Not only does this reduction take place in ants in such wet surroundings at low temperatures, but also in ants kept active at a fairly constant moderate temperature and merely moist surroundings, for laboratory ants maintained all winter at 20°–25° C. and surrounded with moist soil also showed a reduction in water content. The loss of water in the laboratory ants was not great, however, and returned to normal in January. Such water-content behavior must be the result of cyclic changes in metabolism, and not merely temperature and humidity effects, as determined for oak-borers (Payne, 1926). It is with metabolic water that we are concerned in the case of these ants.

There is nothing unusual about the body-temperature phenomena. The ants react as most cold-blooded animals do when subjected to low temperatures. Unlike the honey bee, this species of Hymenoptera gives no evidence of any appreciable heat production or conservation in winter. Whether it is capable of maintaining a constant nest temperature in summer, such as is reported for *Formica rufa* (Steiner, 1924), has not been determined.

Of course, there is no feeding in nature during the winter, since these ants are so sluggish and so closely packed in their burrows that feeding would be out of the question even if food were available.

There is undoubtedly a reduction in body weight as a result of the loss of water and of the consumption of stored food in the body, though this point was not specifically investigated.

The advantages accruing from the type of hibernation seen in this species are numerous. The ants are fully protected from freezing, not only by their retirement far below the frost line, but also by the increased concentration of their body fluids as a result of the loss of water. Even those that over-winter in the upper frozen parts of the nest are none the worse for their exposure because of this lowering of the freezing-point and because of the insulating effect of the snow and ice covering the mound. Should the surface temperature of the mound descend considerably below 0.0° C., the ants are further protected by the well-known super-cooling phenomena (Bachmetjew, 1900; Payne, 1926) of the body fluids. The reduction in metabolism obviates the necessity for the intake of food;

whatever nourishment is required is amply provided by the fat body. The reduction in gaseous exchange prevents suffocation while aggregated into the densely packed masses noted. The reduction in the O_2 requirement also permits submergence for a longer period without injury, should this happen. The maintenance of a small amount of activity allows them to adjust their position to the fluctuating water level and to respond to other strong stimuli. The benefit of aggregation in winter is not clear. There is no appreciable heat conservation or production in the aggregation, and there is no special need for this since they are so well protected against freezing by other means. It is possible that there is a mechanical protection against drowning when the water rises by the trapping of bubbles of air among the many legs, antennae, and bodies of the ants, so that the ants have time to readjust their position. There may also be a satisfaction of a gregarious instinct. But it may well be that the winter aggregation represents merely an incidental congregation of individuals within more or less limited optimal portions of the nest.

LITERATURE CITED

ALLEE, W. C. 1926. Studies in animal aggregations: Causes and effects of bunching in land isopods. Jour. Exp. Zoöl., XLV, 255–77.

———. 1927. Animal aggregations. Quar. Rev. Biol., II, 367–98.

———. 1927. Studies in animal aggregations: Some physiological effects of aggregation on the brittle starfish, *Ophioderma brevispina*. Jour. Exp. Zoöl., XLVIII, 475–95.

ALLEE, W. C., and SCHUETT, J. F. 1927. Studies in animal aggregations: The relation between mass of animals and resistance to colloidal silver. Biol. Bull., LIII, 301–17.

ATHANASIU, J. 1909. Hibernation. Richet's Dict. d. Physiol., VIII, 563–623.

BABCOCK, S. M. 1912. Metabolic water: Its production and rôle in vital phenomena. Univ. of Wis. Agr. Exp. Sta. Res. Bull. No. 22.

BACHMETJEW, P. 1900. Freezing of body fluids in insects. Zeitsch. Wiss. Zoöl., LXVII, 529–50.

BAUMBERGER, J. P. 1914. Studies in the longevity of insects. Ann. Ent. Soc. Amer., VII, 323–53.

———. 1917. Hibernation: A periodic phenomenon. *Ibid.*, X, 179–86.

BODINE, J. H. 1921. Factors influencing the water content and the rate of metabolism of certain Orthoptera. Jour. Exp. Zoöl., XXXII, 137–64.

———. 1923. Hibernation in Orthoptera. I. Physiological changes during hibernation in certain Orthoptera. *Ibid.*, XXXVII, 457–76.

BREITENBECHER, J. H. 1918. Relation of water to behavior of the potato beetle in a desert. Carnegie Inst. Pub. No. 263.

COBELLI, RUGERO. 1903. L'ibernazione delle formiche. Verh. d. k. k. Zoöl.-Bot. Gesellschaft (Wien), LIII, 369–80.

FINK, DAVID E. 1925. Physiological studies on hibernation in the potato beetle, *Leptinotarsa decemlineata* Say. Biol. Bull., XLIX, 381–406.

GATES, BURTON N. 1914. Temperature of the bee colony. U.S. Dept. Agr. Bull. No. 96.

GREELEY, A. W. 1901. An analogy between the effects of loss of water and lowering of temperature. Amer. Jour. Physiol., VI, 122–28.

HARRINGTON, GEORGE T., and CROCKER, WILLIAM. 1923. A new and efficient respirometer for seeds and other small objects: Directions for its use. Jour. Agr. Res., XXIII, 101–17.

HILL, A. V. 1911. The total energy exchanges of intact cold blooded animals at rest. Jour. Physiol., XLIII, 379–94.

HINDS, W. E., and YOTHERS, W. W. 1909. Hibernation of the Mexican cotton boll weevil. U.S. Dept. Agr., Bur. of Ent., Bull. No. 77.

HOLMQUIST, A. M. 1926. Studies in arthropod hibernation. I. Ecological survey of hibernating species from forest environments of the Chicago region. Ann. Ent. Soc. Amer., XIX, 395–426.

———. 1928. Notes on the life history and habits of the mound-building ant, *Formica ulkei* Emery. Ecol., IX, 70–88.

KENNEDY, CLARENCE H. 1927. Some non-nervous factors that condition the sensitivity of insects to moisture, temperature, light and odors. Ann. Ent. Soc. Amer., XX, 87–106.

KROGH, AUGUST. 1915. Respiratory exchange of animals and man. New York: Longmans, Green and Co.

———. 1923. The respiration of insects. Jour. Wash. Acad. Sci., XIII, 160–62.

LANG, ARNOLD. 1899. Ueber den Saisonschlaf der Tiere. Schweiz. Paedagog. Zeitsch., IX, 289–305.

MCCOLLOCH, J. W., and HAYES, WILLIAM P. 1923. Soil temperature and its influence on white grub activities. Ecol., IV, 29–36.

MCCOOK, HENRY C. 1877. Mound-making ants of the Alleghenies.—Their architecture and habits. Trans. Amer. Ent. Soc., VII, 253–96.

PARHON, MARIE. 1909. Les échanges nutritifs chez les abeilles pendant les quatre saisons. Ann. d. Sci. Nat., Zoöl., Series 9, IX, 1–59.

PARKER, H. L., and THOMPSON, W. R. 1927. Contribution to the study of hibernation in the larva of the European corn borer (*Pyrausta nubilalis* Hubn.). Ann. Ent. Soc. Amer., XX, 10–22.

PAYNE, NELLIE M. 1926. Freezing and survival of insects at low temperature. Quar. Rev. Biol., I, 270–82.

PHILLIPS, E. F., and DEMUTH, GEORGE. 1914. Temperature of honey bee cluster in winter. U.S. Dept. Agr. Bull. No. 93.

PIRSCH, GREGOR B. 1923. Studies on body temperature of the honey bee. Jour. Agr. Res., XXIV, 275–88.

PLATH, O. E. 1927. Notes on the hibernation of several North American bumblebees. Ann. Ent. Soc. Amer., XX, 181–92.

RASMUSSEN, A. T. 1916. Theories of hibernation. Amer. Nat., L, 609–26.

ROBINSON, WILLIAM. 1927. Water binding capacity of colloids a definite factor in winter hardiness of insects. Jour. Econ. Ent., XX, 80–88.

———. 1927. The thermocouple method of determining temperatures. Ann. Ent. Soc. Amer., XX, 513–21.

ROUBAUD, E. 1922. Études sur le sommeil d'hiver pre-imaginal des muscides. Bul. Biol. de la France et la Belgique, LVI, 455–544.

———. 1922. Sommeil d'hiver cédant à l'hiver chez les larves et nymphes de muscides. Compt. Rend. Acad. d. Sci., CLXXIV, 964–66.

SANDERSON, E. DWIGHT. 1908. Relation of temperature to the hibernation of insects. Jour. Econ. Ent., I, 56–65.

SCHMIDT, PETER. 1918. Anabiosis of the earthworm. Jour. Exp. Zoöl., XXVII, 57–72.

SHERMAN, HOPE. 1921. Respiration of dormant seeds. Bot. Gaz., LXXII, 1–30.

STEINER, A. 1924. Ueber den sozialen Waermehaushalt der Waldameise, Formica rufa var. rufo-pratensis For. Zeitschr. vergl. Physiol., II, 23–56.

TANQUARY, MAURICE COLE. 1913. Biological and embryological studies on the Formicidae. Bull. Ill. State Lab. Nat. Hist., IX, Art. 9, 417–79.

TOWER, W. L. 1906. An investigation of evolution in chrysomelid beetles of the genus *Leptinotarsa*. Carnegie Inst. Pub., Wash., No. 48.

VERNON, H. M. 1897. Relation of the respiratory exchanges of cold-blooded animals to temperature. Part II. Jour. Physiol., XXI, 443–96.

WHEELER, WILLIAM M. 1926. Ants. Columbia University Press.

WHITE, W. P. 1910. The thermoelement as a precision thermometer. Phys. Rev., XXXI, 135.

THE EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF AN INSECT (POPILLIA JAPONICA NEWMAN)

(Nine figures)

DANIEL LUDWIG
Zoölogical Laboratory, University of Pennsylvania

INTRODUCTION

MANY investigators of temperature and its relation to growth and development consider growth as a chemical process. Attempts are made to interpret the differences in rates of development associated with different temperatures by applying the rules governing chemical reactions. According to van't Hoff's hypothesis (1884), the rates of chemical reactions are either doubled or tripled for each 10° rise in temperature on the centigrade scale. This is expressed as the constant Q_{10}. Hertwig (1898) was among the first to make a systematic and quantitative study of development at different temperatures and to apply the van't Hoff formula, obtaining a Q_{10} value of more than 2. The value of Q_{10} has been determined for a number of biological processes; and whenever more than 2 is obtained, it is concluded that the process is chemical in nature. Snyder (1911) made a study of Q_{10} values obtained for various biological processes, and showed that they are not constant but decrease as the temperature is increased. Krogh (1914) showed that the Q_{10} values obtained for developing frogs' eggs are not constant but also decrease as the temperature is increased. He showed that the increase in rate of development is in direct proportion to the increase in temperature and can be more accurately expressed by adding a constant, K_{10}, for each 10° C. rise in temperature within certain limits, usually called the limits of normal development. He made no attempt to analyze growth in terms of either chemical or physical processes. Crozier (1924) employed the formula of Arrhenius (1915)

$$\frac{K_2}{K_1} = e^{\mu\left(\frac{1}{T_1} - \frac{1}{T_2}\right)},$$

where K_1 is the rate at one temperature, K_2 is the rate at another temperature, e is the base of natural logarithms, μ is a constant, and T_1 and T_2 are the temperatures used, expressed on the absolute temperature scale. According to this method, growth and development are recognized as the result of a complex series of chemical reactions whose rates are governed by the rate of the slowest or "master" reaction. The method is an attempt to analyze the kind of reaction underlying the various complex processes involved, since μ is constant for reactions determined by the same catalyst. Friend (1927) recently applied the Arrhenius formula to the development of the birch-leaf skeletonizer *Bucculatrix canadensisella* and obtained diminishing values of μ as the temperature was increased.

Many of the investigations on the influence of temperature on development deal with only one stage of the life-cycle, such as the egg or the pupa. The question may be asked, "Do all the stages in the life-cycle of an insect react in a similar manner, so that results obtained on one may be used in an interpretation of the reactions of the other stages?" Usually records are made of groups of animals without any consideration of how the individuals composing the groups respond. Frequently, investigators discard from their records those results which are widely different from the average of the group. This is often unjustifiable and might lead to a neglect of facts which may be important in understanding the true relations existing between environmental factors and development.

The discussion which follows deals with the development of the various stages in the life-cycle of the Japanese beetle under controlled temperature conditions. The development of each individual is recorded from the time the egg is laid until the animal emerges as an adult. The results are analyzed according to each of the foregoing methods. Since under field conditions the beetles are subjected to daily varying, rather than to constant, temperatures, experiments were also performed to study the effect of daily alternation of temperatures as compared with the effect of a constant temperature corresponding to their mean value.

ACKNOWLEDGMENTS

The work on this problem was begun while the writer was in the employment of the Japanese Beetle Laboratory, United States

Department of Agriculture, Moorestown, New Jersey. He is indebted to Dr. Henry Fox, of the Japanese Beetle Laboratory, for suggesting the problem, supplying material, and giving helpful suggestions throughout the investigations.

The experiments were performed at the Zoölogical Laboratory, University of Pennsylvania, and the writer wishes to express his appreciation to Dr. C. E. McClung, director of the Laboratory, for placing at his disposal the necessary equipment. The writer is especially grateful to Dr. J. H. Bodine for valuable advice and criticism.

MATERIAL AND METHODS

Throughout the experiments, daily observations and individual records were made. Individuals of each stage, except the egg stage, were placed separately in 1-ounce metal salve boxes. These boxes had been previously sterilized by heating in a hot-air sterilizer for 30 minutes at a temperature of 150°–200° C. Moist plant mold which had been fumigated by treatment with carbon bisulphide was placed in each box. The eggs were also kept in sterile 1-ounce salve boxes but were placed in depressions in moist sterile soil. The humidity was maintained approximately constant (near the point of saturation) by the addition of tap water to the soil or plant mold.

To study the rate of embryonic development at constant and alternating temperatures, a large number of eggs were collected during the summers of 1926 and 1927 from beetles kept in breeding-cages in the laboratory. The soil in the cages was sifted daily so that the eggs, when obtained, were less than 24 hours old. They were divided into series, some of which were used for constant, and others for alternating, temperature conditions. A series was placed at each of the following constant temperatures (expressed in degrees centigrade): $10° \pm 1°$; $13° \pm 0.5°$; $15° \pm 1°$; $17.5° \pm 1°$; $20° \pm 1°$; $22.5° \pm 0.5°$; $25° \pm 0.5°$; $27.5° \pm 1°$; $30° \pm 1°$; $31° \pm 0.5$; $33° \pm 0.5°$; $34° \pm 0.5°$; and $35° \pm 0.5°$. A series was also placed at each of the following alternating temperature conditions: 10 and 20°; 15 and 25°; 20 and 30°; 25 and 35°. For example, by "10 and 20°" is meant that a series was placed one day at 10°, the next day it was removed from 10° and placed at 20°, and the following day returned to 10°, and so on. An effort was made to transfer each series at the same

time each day. No changes were made on Sundays, corrections being made in the calculations of the mean temperature for each series. The number of eggs used at each temperature is given in Table I.

The larvae, as soon as hatched, were transferred from the boxes containing soil to boxes containing moist plant mold and, with the

TABLE I

NUMBER OF INDIVIDUALS USED IN EXPERIMENTS AT EACH TEMPERATURE

TEMPERATURE (C.)	EGGS	LARVAE			PUPAE
		First Instar	Second Instar	Third Instar	
40°	.....	.....	.....	.....	65
37°	.....	.....	.....	.....	55
36°	.....	.....	.....	.....	59
35°	100	45	.....	.....	68
34°	78	.....	.....	.....	.....
33°	93	30	.....	.....	.....
32.5°	.....	.....	.....	.....	70
31°	78	26	24	.....	.....
30°	100	87	54	30	70
27.5°	197	131	111	86	72
25°	359	243	192	160	155
22.5°	100	99	65	61	128
20°	273	210	146	126	167
17.5°	102	116	39	.....	96
15°	50	45	15	.....	64
13°	103	.....	.....	.....	54
10°	50	70	30	.....	39
Alternating temperatures:					
30° and 40°	.....	.....	.....	.....	29
25° and 35°	98	58	.....	.....	37
20° and 30°	100	82	68	61	74
15° and 25°	103	83	67	61	83
10° and 20°	100	73	25	19	41
10° and 15°	.....	.....	.....	.....	57
Total	2,084	1,398	836	604	1,483

few exceptions mentioned below, were continued under the same temperature conditions as the eggs from which they hatched. That is, larvae hatched from eggs which had been kept at 20° C. were also kept at 20° C until the adults emerged. In this way daily records were obtained of the length of the entire developmental cycle at each of the temperatures used at which complete development was possible. At 10° C. no larvae were obtained; at 15° C. the eggs hatched but the larvae did not survive; at 17.5° C. their mortality was very great. To determine if they were able to survive and de-

velop at these relatively low temperatures, eggs were hatched at 25° C., and the larvae hatched from these eggs were transferred to 10° 15°, and 17.5° C. To ascertain if second-instar larvae were also able to survive and develop at 10° and 15° C., larvae which had been allowed to develop to the first molt at room temperature (22°–27° C.) were transferred to these lower temperatures.

The larval period of the Japanese beetle consists of three instars, the last of which includes a prepupal period during which the larva ceases feeding, the food material and fecal matter is discharged from the alimentary canal, the mouth parts and legs become immotile, and the body shrinks within the larval skin. As soon as this skin splits on the dorsal surface, the animal is called a pupa. Records were made of each of the three instars; but since there is no well- defined external change which marks the transformation of the prepupa from the third-instar larva, this larval period is calculated from the second until the third molt. In respect to food conditions, the experiments were divided into (1) larvae which were fed on only plant mold during the first and part of the second instar, when wheat was also included in the diet, and (2) larvae which were fed on plant mold and wheat throughout the entire larval period. Food conditions were controlled by keeping an abundance of food available for the larvae throughout the experiments. A list of the constant and alternating temperatures used in the experiments on the larval period, as well as the number of larvae used at each temperature, is given in Table I.

Pupae were obtained from two different sources: those which had developed from the larvae used in these experiments, and those developed from prepupae collected in the field during the months of June, 1926, and June, 1927. The experiments on the prepupae collected in the field were as follows: The prepupae were divided into series and kept at room temperature (22°–27° C.) until they molted to pupae. The pupae obtained from each series were then placed at one of the following constant temperatures (expressed in degrees centigrade): $10° \pm 1°$; $13° \pm 0.5°$; $15° \pm 1°$; $17.5° \pm 1°$; $20° \pm 1°$; $22.5° \pm 0.5°$; $25° \pm 0.5°$; $27.5° \pm 1°$; $30° \pm 1°$; $32.5° \pm 1°$; $35° \pm 0.5°$; $36° \pm 0.5°$; $37° \pm 0.5°$; and $40° \pm 0.5°$. Other series of pupae thus obtained were placed at one of the following alternating temperature conditions;

10 and 15°; 10 and 20°; 15 and 25°; 20 and 30°; 25 and 35°; 30 and 40°. The number of individuals used at each temperature is given in Table I.

RESULTS

Effects of constant temperatures.—A study of the effects of temperature on the development of different stages in the life-cycle of the Japanese beetle shows that there is a different threshold of development for the egg, larval, and pupal stages. The time required is longer at low temperatures and decreases with higher temperatures until an optimum point is reached. Above this point, it is again increased until the temperature reaches the maximum at which development is possible.

Figure 1 shows that for the egg the time varies from 7.98 ± 0.018 days at 30° C. to 61 ± 0.68 days at 15° C. No eggs hatched at any temperatures used below 15° C.; although at 13° C. some development took place, as shown by the appearance of the mandibles of the larvae through the egg membrane. Above 30° C. the time was again increased to 9 days at 34° C.

The following discussion of the larval period deals only with larvae fed on plant mold during the first instar and part of the second instar, when wheat was also included in the diet. Results were obtained for the total larval period at temperatures between 20° and 27.5° C. Above and below these points the larvae did not transform to pupae. The time required for the total larval period is not influenced greatly by temperature. For example, at 25° C. the average time was 149.2 days, and at 20° C. it was 160.3 days—a difference of only 11 days. However, if the time of development of each of the three instars is considered separately, it is found that the length of each is not only dependent on temperature and food but also on a time factor, which will be discussed later. As shown in Figure 2, the first instar larval period varies from 16.9 ± 0.25 days at 30° C. to 160 days at 15° C. At 17.5° C. it required 101.4 ± 1.6 days; while at 20° C. only 37.6 ± 1.1 days. The time at 17.5° C. is longer than expected on the basis of the time-temperature curve obtained at the higher temperatures. Some factor other than temperature and food must, therefore, influence development at this temperature. This fact becomes more evident when the results obtained

at 20° C. are examined. Here the larvae fall into two groups, one of which passes the first instar in an average time of 30 days and the other in the average time of 80 days. In the first group the time

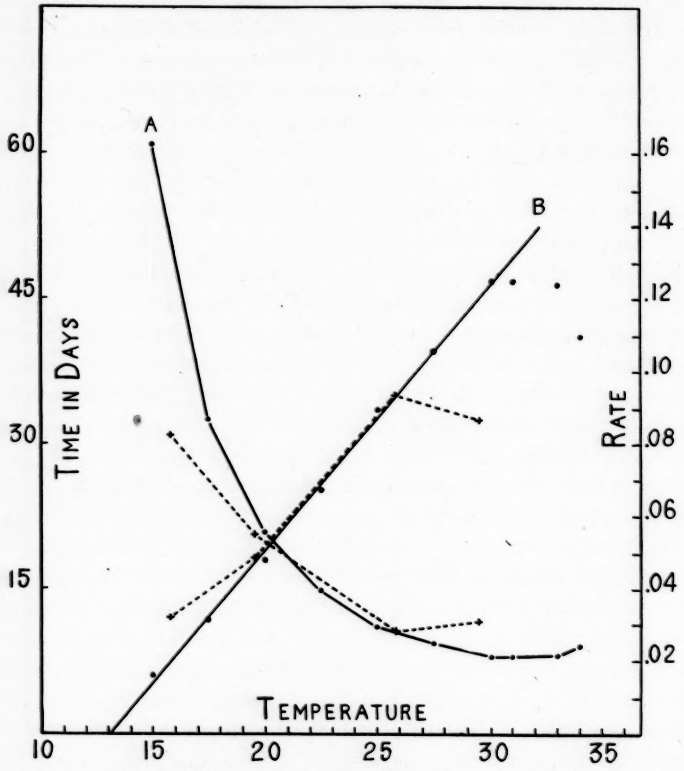


FIG. 1.—Influence of temperature on the development of the egg. *A*, time curve; *B*, rate curve. The solid line represents constant-temperature experiments, while the broken line represents alternating-temperature experiments.

varied from 24 to 48 days, while in the second group it varied from 70 to 101 days.

The length of the second instar larval period was found to depend not only on temperature and food but also on the length of the first instar. Figure 3 shows that it varied from 30.7 ± 0.43 days

at 27.5° C. to 210.5 ± 4.5 days at 15° C. (In this figure the length of the second instar at 15° is not plotted, since it is based on larvae hatched and allowed to develop to the first molt at room temperature,

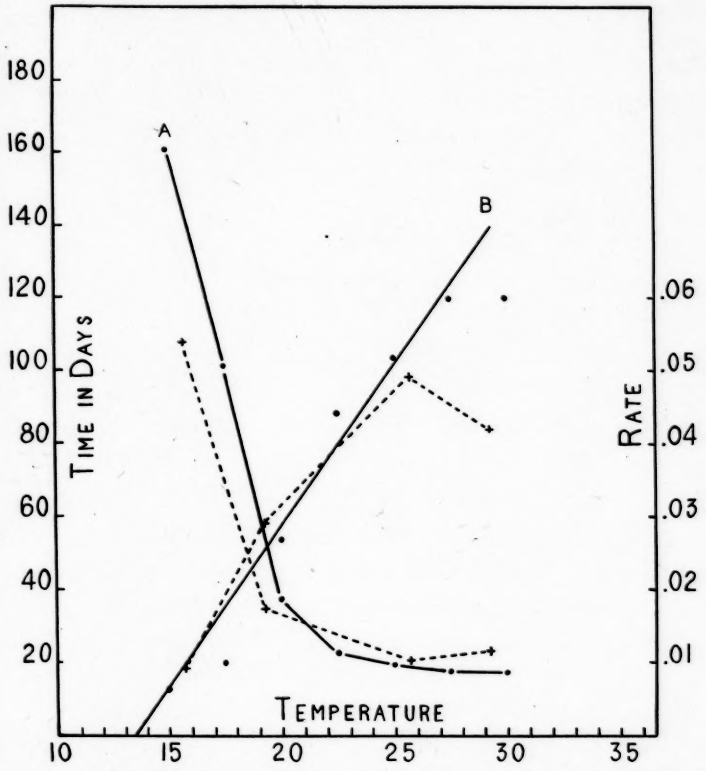


FIG. 2.—Influence of temperature on the development of the first-instar larva. *A*, time curve; *B*, rate curve. The solid line represents constant-temperature experiments, while the broken line represents alternating-temperature experiments.

those obtained at 15° C. having died before completing the second instar.) At 20° C. those individuals which had a short first have a long second instar period, while those which had a long first have a short second instar period. In both groups, the sum of the first and second instars is approximately 108 days. At 22.5° C. the second

instars also fall into two groups, one with a relatively short and the other with a relatively long second instar. In this case there was no sharp demarcation between the individuals of each group.

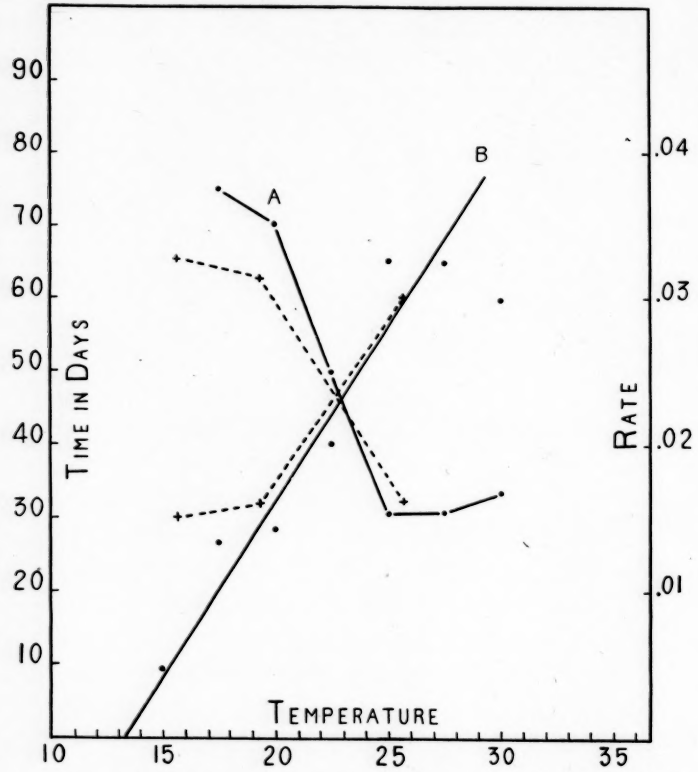


FIG. 3.—Influence of temperature on the development of the second-instar larva. *A*, time curve; *B*, rate curve. The solid line represents constant-temperature experiments, while the broken line represents alternating-temperature experiments. The time at 15° was 210 days. This point is not plotted because the larvae were kept at room temperature (22°–27° C.) until the beginning of the second instar. It is therefore not comparable to the other results plotted.

The time required for the development of the third instar (Fig. 4) was found to be more dependent upon the past history of the larva than any other factor. At 25° C. it was relatively long, being

99.3 ± 1.8 days; at 22.5° C. it was 75.2 ± 2.1 days; at 20° C. it was relatively short, being 52.4 ± 1.6 days. At 22.5° C. those individuals which had a short second have a long third, while those which had

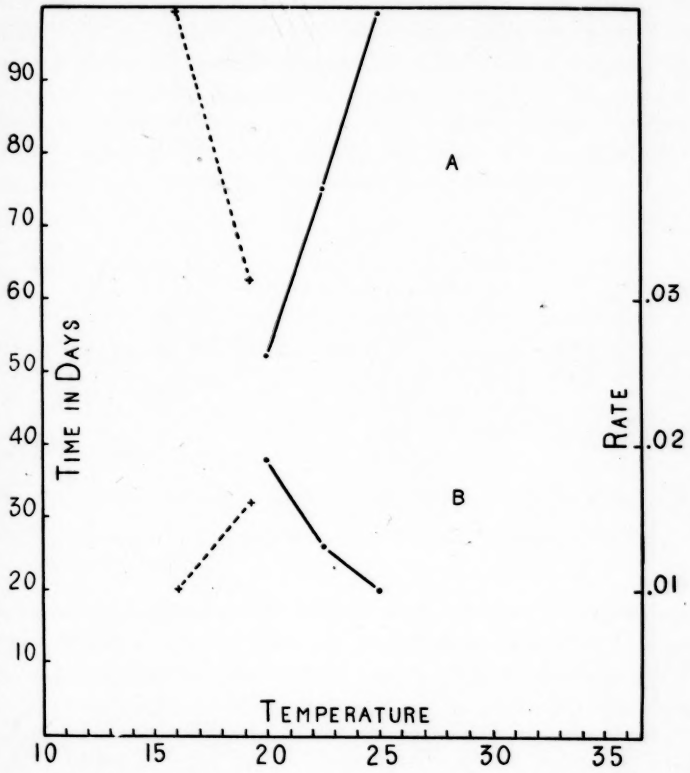


FIG. 4.—Influence of temperature on the development of the third instar larva. *A*, time curve; *B*, rate curve. The solid line represents constant-temperature experiments, while the broken line represents alternating-temperature experiments.

a long second have a short third instar. As a result, the sum of the second and the third instars is also approximately constant at this temperature. These results are shown graphically in Figure 7.

The observation that the third instar period is longer at higher than at lower temperatures led to an experiment to determine if

this stage actually does develop faster at low than at high temperatures or if its length is entirely dependent upon the past history of the individual. Eggs were collected in the laboratory and divided into four series. Series 1 was placed at 25° C. and was kept there until adults emerged. Series 2 was also placed at 25° C. until the larva molted to the third instar, when they were immediately transferred to 20° C. Series 3 was placed at 20° C. and was kept there until the adults emerged. Series 4 was placed at 20° C. until the larva molted to the third instar, when they were transferred to 25° C. In each series the larvae were fed both wheat and plant mold

TABLE II

EFFECT OF TEMPERATURE ON THE THIRD INSTAR WHEN THE PAST HISTORY OF THE LARVA HAS BEEN CONTROLLED

Series	No. of Larvae	First Instar	Second Instar	Third Instar	Total Larval Stage
1.....	50	25° C. 17.4±0.16 days	25° C. 16.9±0.3 days	25° C. 104.8±1.8 days	139.1 days
2.....	45	25° C. 17.9±0.27 days	25° C. 17.2±0.27 days	20° C. 136.6±1.4 days	171.7 days
3.....	45	20° C. 28.1±0.54 days	20° C. 80.5±1.2 days	20° C. 61.9±3.1 days	170.5 days
4.....	50	20° C. 29.9±0.79 days	20° C. 80.8±1.18 days	25° C. 39.4±0.8 days	150.1 days

throughout the entire larval period. The results summarized in Table II show that the length of the third instar is dependent principally upon the lengths of the other larval stages but is also influenced by temperature. In this experiment the past history of the larva as a factor in determining the length of the third instar has been eliminated, leaving temperature as the only factor to be considered. In Series 1 and 2, the first and second instars are both short and the third is long; while in series 3 and 4, the second is long and the third is relatively short. In the former case, the third instar is 31.8 days longer at 20° than at 25° C., while in the latter, the difference is 22.5 days.

The pupal stage is very regular in its response to temperature changes, always developing more slowly at low than at high temperatures (between 15° and 32.5° C.) regardless of the past history

of the individual. Results with pupae which were obtained from prepupae collected in the field were the same as those with pupae which had developed under laboratory conditions, although the for-

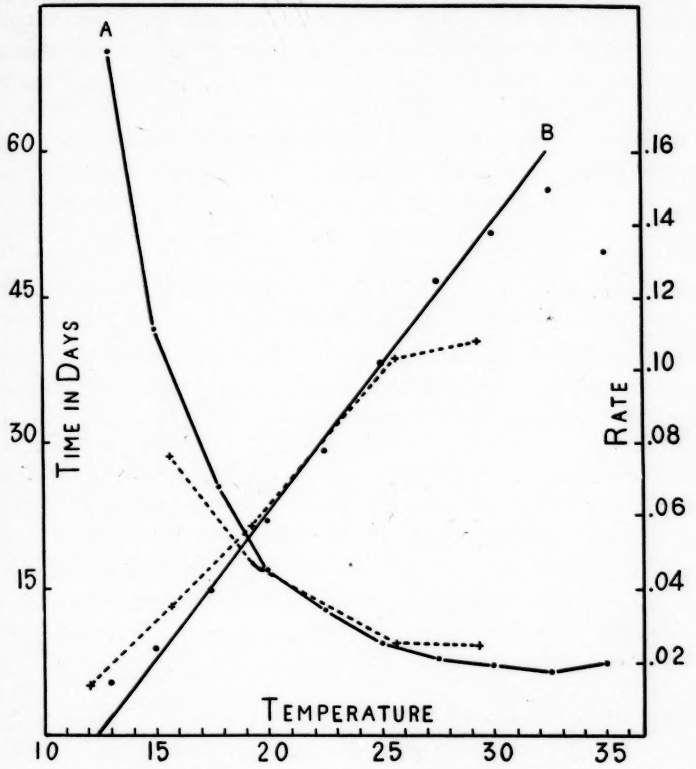


FIG. 5.—Influence of temperature on the development of the pupal stage. *A*, time curve; *B*, rate curve. The solid line represents constant-temperature experiments, while the broken line represents alternating-temperature experiments.

mer had passed the winter as larvae in the field at low temperatures while the latter were kept at controlled high temperatures. The length of the pupal stage (Fig. 5) ranges from 6.6 ± 0.04 days at 32.5° C. to 70.4 ± 0.49 days at 13° C. Above 32.5° C. the time is again increased to 7.5 ± 0.082 at 35° C. Figure 6 shows a comparison

of the time required for the development of each stage at the different temperatures used in these experiments.

Effects of food on larval development.—The experiments on the effects of food on the development of the larva are not sufficiently

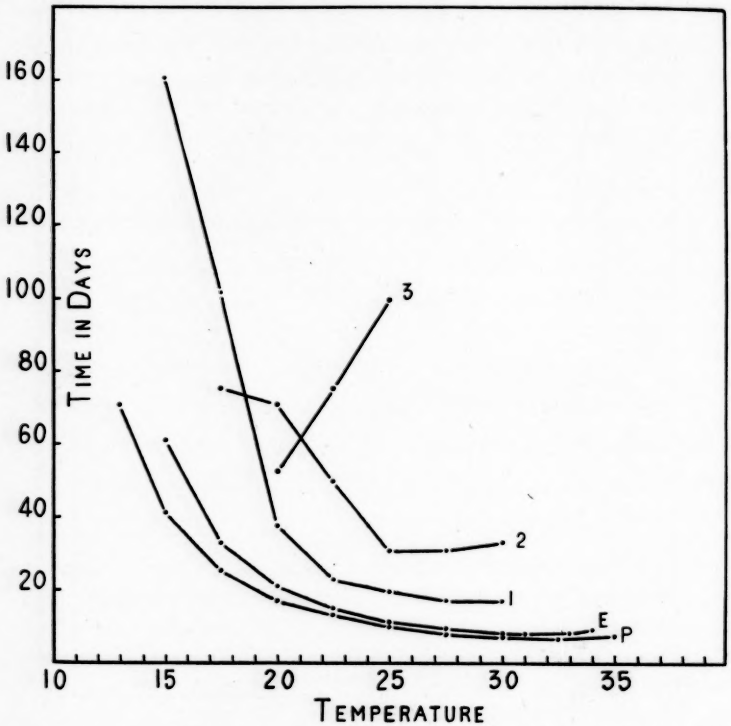


FIG. 6.—Comparison of the time required for the development of each stage. *P*, pupal stage; *E*, egg; (1) first-instar larva; (2) second-instar larva; and (3) third-instar larva.

complete to justify a detailed analysis of results, although a few preliminary statements may be made. The results seem to show that the presence of wheat in the diet hastens the development of those stages not affected by the time factor described above. However, the stage affected by this time factor seems to be correspondingly

longer so that the length of the total larval period is not affected. This can be seen by comparing the results shown in the time-temperature curves for larval development in Figures 2, 3, and 4 with those in Table II, and also by studying the chart of the total life-cycle in Figure 7. The presence of wheat in the diet also seems to reduce the number of individuals at 20° C. which are retarded in the first instar (Fig. 7). At 17.5° C. the first instars of those individuals fed only on plant mold were retarded in each case, while those fed on wheat and plant mold were divided into two groups, some with a long and others with a short first instar. These results may be due to the hastening of development just described. Larvae fed wheat and plant mold seem to be more resistant to extreme temperature conditions than those fed only on plant mold. For example, at 27.5° C. only one pupa was obtained from larvae fed on plant mold although 88 first instar larvae were placed at that temperature. On the other hand, 5 pupae were obtained from 43 larvae fed wheat and plant mold and kept at that temperature.

Toleration of extreme temperatures by various stages.—The experiments at the upper and lower temperature extremes led to a number of interesting observations. Eggs will hatch at temperatures which are too low or too high to permit the larvae, which hatch from them, to survive. Seventy-five per cent of the eggs placed at 33° hatched, but the larvae died within a few days. At 15° C. only 42 per cent of the eggs hatched, the larvae also dying within a few days. However, larvae which hatched at room temperature (22°–25° C.) and were transferred immediately to 15° C. were able to live at that temperature, some developing into second instars, all of which died soon after molting. Second-instar larvae which had hatched and developed to the first molt at room temperature, were able to survive at 15° C. At 17.5° C., 79 per cent of the eggs hatched, but only 21 per cent of the larvae reached the second instar, while 76 per cent of the larvae hatched at 25° C. and, transferred the same day to 17.5° C., were able to develop to the second instar.

The highest temperature at which normal adults were obtained was 35° C. Although pupae placed at 36° C. seemed to develop normally, the adult structures forming within the pupal skin, and the color of the head, thorax, and legs changing to the dark green char-

acteristic of normal pupae just before emergence, no adults were obtained. At 37° C. the pupae lived from 1 to 5 days but never

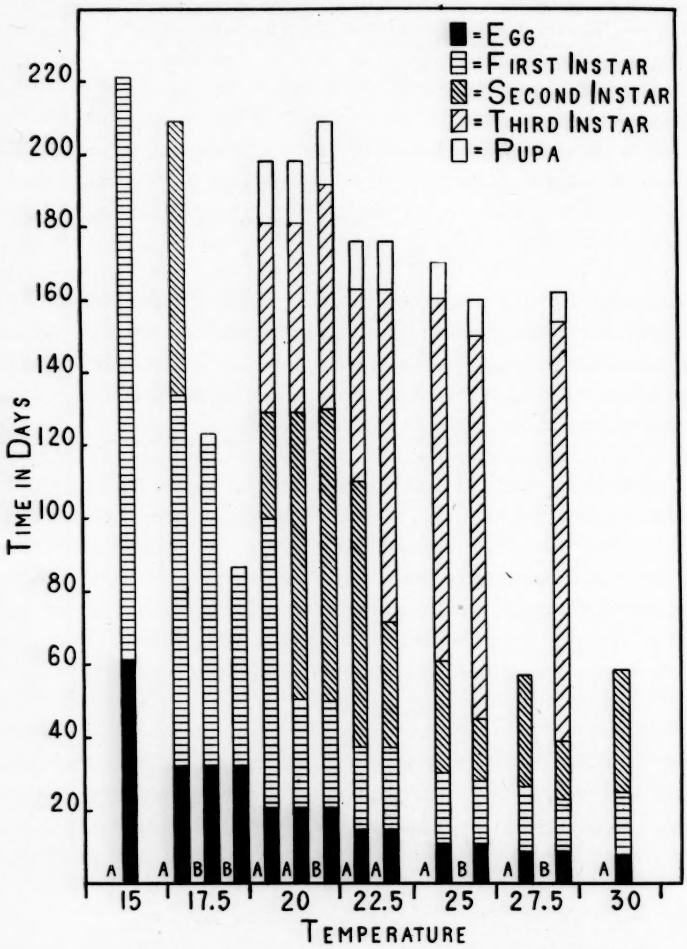


FIG. 7.—Relative lengths of each stage of the life-cycle at different temperatures. *A*, those individuals fed on plant mold; *B*, those fed on plant mold and wheat during the entire larval period. The larvae represented at 15° and at 17.5° (*A*) were not hatched from the eggs kept at those temperatures.

showed any external signs of development. At both extremes of temperature (13° and 35° C.) at which normal adults were obtained, there were also many abnormal individuals which were unable to shed the pupal skin entirely. In these the abdomen failed to diminish in size (as in the normal adult), the wings did not unfold, and the animal resembled a pupa, except that it was able to move its legs and mouth parts.

Effects of alternating temperatures.—The time required for the development of any stage at alternating temperature conditions, compared with the time at a constant temperature which corresponds to the mean of the alternating temperatures, seems to indicate that the effects depend on the temperatures involved. (1) If one of the alternating temperatures is above the optimum of development and the other is between the threshold and the optimum, development is slower than at the mean constant temperature. For example, Figure 1 shows that eggs placed at the alternating temperatures of 25° and 35° C. required 11.5 ± 0.05 days to hatch, while those placed at a constant temperature of 30° C. required only 7.98 ± 0.018 days. Under the same temperature conditions, the first-instar larvae required 23.3 and 16.9 ± 0.25 days (Fig. 2); the pupae required 9.2 ± 0.118 and 7.2 ± 0.054 days (Fig. 5). No readings were made at these alternating temperatures for the second- and third- instar larvae. (2) If both of the alternating temperatures are between the threshold and the optimum, development is neither retarded nor accelerated, as compared with the mean constant temperature. Eggs placed at the alternating temperatures of 15° and 25° C. required 20.4 ± 0.062 days to hatch, while those kept at a constant temperature of 20° C. required 20.9 ± 0.048 days. Under the same temperature conditions, the first-instar larvae required 34.1 ± 0.64 and 37.6 ± 1.1 days; the second-instar larvae required 62.6 ± 1.3 and 70.3 ± 1.56 days; the third-instar larvae required 62.8 ± 1.48 and 52.4 ± 1.6 days; and the pupae required 17.5 ± 0.102 and 17.25 ± 0.071 days. In this case, the first- and second-instar larvae seem to develop faster at the alternating temperatures than at 20° C.; but if the entire larval period be calculated, it is found to be 159.5 and 160.3 days. (3) If one of the alternating temperatures is below the threshold and the other is between the threshold and the optimum,

development seems to be accelerated, as compared with the mean constant temperature. Eggs placed at the alternating temperatures of 10° and 20° C. required 31.15±0.158 days to hatch; while those placed at 15° C. required 61±0.68 days. Under the same temperature conditions, the pupae required 28.6±0.24 and 41.6±0.22 days. Figure 2 shows that the rate of development of the first instar at 10° and 30° C. is not accelerated, as compared with the rate curve obtained for constant temperatures. Undoubtedly, the reason is that under these conditions, development is retarded in the first instar by the time factor, the second and third being correspondingly shortened, as shown in Figures 3 and 4. Comparable readings were not obtained for the second- and third-instar larvae at 15°.

DISCUSSION

Effects of temperature on different stages of the life-cycle.—The results just described show that the different stages of the life-cycle of the Japanese beetle are not affected to the same degree by temperature. The threshold of development differs for the egg, larva, and pupa. To illustrate, pupae transformed to normal adults at 13°, but no eggs hatched at that temperature. Figure 8 shows that the rates of development $\left(\frac{1}{\text{time}}\right)$ of each stage are not accelerated to the same extent by an increase in temperature. The rate of development of the egg and of the pupa is accelerated to a greater extent than that of the larva. The optimum temperature for development also differs with each stage. The optimum for the pupa is between 30° and 32° C.; that for the egg is approximately 30° C., while that for the larva is approximately 27.5° C. Each stage has a different maximum temperature at which development is possible. Pupae transformed to normal adults at 35° C., but no eggs hatched above 34° C., and no larvae transformed to pupae above 27.5° C. Sanderson (1910) discussed the relation of temperature to the growth of insects particularly in respect to threshold temperatures and thermal constants of development in different species and in different stages of the life-history of the same species. He showed that each species of insect has its own threshold temperature and that each stage in the life-cycle may react differently from the other stages, the effect

on the life-cycle being the sum total of the effects on each stage. Bonnier (1926) showed that in *Drosophila melanogaster* an increase of temperature has a different effect on different stages and that it shortens the time during pupation relatively more than the

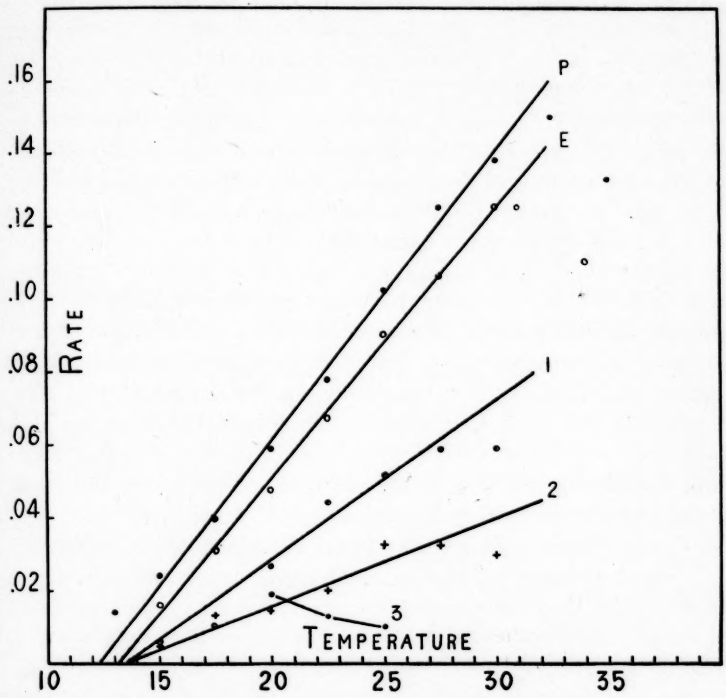


FIG. 8.—Comparison of the rates of development of each stage. *P*, pupal stage; *E*, egg; (1) first-instar larva; (2) second-instar larva; and (3) third-instar larva.

time up to pupation. Shelford (1927) showed that the threshold of development in the codling moth varies with the individual, and also that the thermal constants of development are not the same for different stages.

In the Japanese beetle, the egg and pupal stages are very regular in their responses to temperature; but the larval stages are affected by other factors, such as the past history of the individual. This is

probably associated with the fact that the larval stages, principally the second and third instars, are the over-wintering stages, and under field conditions development is inhibited completely during the winter. Under laboratory conditions with a constant temperature favorable for development, the larva apparently is forced to go through a resting condition, similar to the over-wintering condition in the field, before it can complete development. An examination of Figure 7 shows that at 25° C. this resting stage always occurs in the third instar; at 22.5° C. it may occur either in the second or third; at 20° C. it occurs either in the first or second, but usually in the second; at 17.5° C. it almost always occurs in the first but may occur in the second. This suggests that there is a time cycle or rhythm in development and that the rate of development, which is dependent on the temperature, determines in which stage the resting period will occur. Bonnier (1926) showed that in *Dorsophila melanogaster* the rate of development of the pupa seems to depend not only on the temperature but also on the length of the prepupal stages. He found that in those individuals which required a relatively long time for development up to pupation, this time was compensated by a shorter time during the pupal stage; also, that if the time for development up to the pupal stage was short, the pupal stage was correspondingly longer.

Frequently investigators have studied temperature effects on development, using only one stage, such as the egg or pupa; and from these results they have made generalizations regarding the effects of temperature on the entire development of the organisms studied. The results obtained by Bonnier on *Drosophila* and by the writer on the Japanese beetle indicate a possible interrelationship between development in different stages. One stage cannot be considered separately unless something is known about the other stages.

Analysis of results obtained at constant temperatures.—(1) Van't Hoff's temperature coefficient as applied to growth and development: Van't Hoff's hypothesis that the rates of chemical reactions are either doubled or tripled for each 10° C. rise in temperature may be calculated from the formula

$$Q_{10}=\left(\frac{K_2}{K_1}\right)^{\frac{10}{t_2-t_1}},$$

where Q_{10} is the temperature coefficient, K is the rate of the reaction at the temperature t_1, and K_2 is the rate of the reaction at the temperature t_2. For many chemical reactions the temperature coefficient is constant for a great range of temperatures, but in some it decreases gradually as the temperature is increased. Van't Hoff suggested that this variation may be due to a change in the viscosity of the solvent associated with the increase in temperature. A number of investigators, Hertwig (1898), Loeb (1908), Loeb and Wasteneys (1911), Krogh (1914), Loeb and Northrop (1917), Krafka (1920) and others, studied the processes of growth and development at various temperatures and calculated the temperature coefficients, obtaining values of more than 2. On this basis,

TABLE III

TABLE OF Q_{10} VALUES

Temperature Range (C.)	Egg	First Instar	Second Instar	Third Instar	Pupa
15°–20°	8.8	19.6	9.0	.....	5.8
17.5°–22.5°	4.7	20.1	2.5	.....	3.8
20°–25°	3.5	3.6	5.1	0.27	3.2
22.5°–27.5°	2.5	1.7	2.5	1.21	2.6
25°–30°	1.9	1.2	0.81	.....	1.8
27.5°–32.5°	1.3	.....	.....	.....	1.4
30°–35°	.....	.....	.....	.....	0.9

Hertwig (1898), Loeb (1908), Loeb and Wasteneys (1911), and Loeb and Northrop (1917) conclude that the processes involved are chemical in nature. On the other hand, Krogh (1914), Krafka (1920), and Chapman (1925) point out that for growth-processes the values of Q_{10} diminish as the temperature is increased and that the change in value is out of proportion for the requirements of van't Hoff's law. Krogh (1914) states, "If the constant is found to vary regularly from the lowest temperatures to the highest it is misleading to speak of it as a constant or to publish the average of a number of determinations over a certain range of temperature as the Q_{10} for the process in question. Such a process does not follow van't Hoff's formula" (p. 165).

The temperature coefficients have been calculated for the development of each stage of the Japanese beetle. The values obtained are given in Table III.

It is very evident that the temperature coefficients diminish quite regularly as the temperature is increased, except in the third instar and in the first and second instars at 17.5° C. The range of variation, for the temperatures at which growth is possible, is so great that one is hardly justified in using an average value as the Q_{10} of the process. Consequently, here also the van't Hoff hypothesis does not apply, and, on this basis, it cannot be assumed that the processes involved are necessarily chemical in nature.

(2) The Arrhenius formula as applied to growth and development: The Arrhenius formula,

$$\frac{K_2}{K_1} = e^{\mu\left(\frac{1}{T_1}-\frac{1}{T_2}\right)},$$

is used to measure the rate of chemical reactions which show a slightly decreasing Q_{10} value as the temperature is increased. In this formula, μ is known as the temperature characteristic, and its values are constant for the same chemical reaction. When the logarithmic value of the rate is plotted against the reciprocal of the absolute temperature, a straight line results. If complex biological processes are governed by a simple chemical reaction (the master reaction), μ may be used as a key to the type of underlying reaction. Rogers (1911) calculated μ for the velocity of the heart beat, obtaining values which were not constant but which decreased as the temperature was increased. Krogh (1914) studied the rate of cleavage of the frog's egg and concluded that the Arrhenius formula does not express the relation any better than van't Hoff's formula. Arrhenius (1915) applied the formula to a few biological processes obtaining fairly constant values of μ. Crozier (1924) analyzed a large number of biological processes by this method and was able to divide them into groups according to their temperature characteristics. Frequently he obtained a break in the curve at certain temperatures. These breaks are explained by assuming that different reactions control the process above than below those temperatures. For many processes he obtained values of 11,500 and 16,700, in which the basic reaction is considered to be probably catalyzed respiratory oxidation. He analyzed the results of Krogh on the development of the frog's egg and obtained values of 10,200 and 22,600. This dif-

ference was explained by assuming that oxidation is not the controlling reaction in growth. Bliss (1926) calculated the temperature characteristics for the prepupal development of *Drosophila melanogaster* and obtained curves which show regularly diminishing values of μ with higher temperatures but which he divided into three straight lines with μ values of 33,000, 16,000, and 7,500. Brown (1926) studied the development of three species of *Cladocera* and obtained values of μ which are of the same magnitude as those obtained by Bliss on *Drosophila* and as those obtained for oxygen consumption in arthropods. Some of his curves also show regularly di-

TABLE IV

VALUES OF μ OBTAINED FOR THE JAPANESE BEETLE

Temperature (C.)	Egg	First Instar	Second Instar	Third Instar	Pupa
13°–15°	.....	.....	.....	.....	44,914
15°–17.5°	43,225	32,707	.....	.....	33,215
17.5°–20°	29,734	66,565	.....	.....	26,065
15°–20°	.....	.....	65,298	.....	.....
20°–22.5°	24,117	35,946	24,462	−27,105	19,934
22.5°–25°	0,352	11,255	33,912	−18,092	19,207
25°–27.5°	11,688	9,175	− 439	.....	14,523
27.5°–30°	12,211	124	−6,175	.....	7,321
27.5°–31°	8,676	.....	.....	.....	.....
30°–32.5°	.....	.....	.....	.....	6,175
30°–33°	− 259	.....	.....	.....	.....
32.5°–35°	.....	.....	.....	.....	−8,909

minishing values of μ with higher temperatures. Friend (1927) studied the development of the birch-leaf skeletonizer *Bucculatrix canadensisella* at different temperatures and obtained values of μ which also decrease as the temperature is increased.

The temperature characteristics have been calculated and the values plotted for the development of each stage of the Japanese beetle. The results are given in Table IV and in Figure 9. A study of the table shows that μ is not constant but is different for nearly every temperature studied. In almost every case the values are higher for lower temperatures, except for the third-instar larva. Correspondingly, Figure 9 shows that there is a break in each curve for nearly every point plotted. It is very evident that if the temperature characteristics can be used as an index to the type of reaction

underlying growth, the reaction is different for each stage of the life-cycle, and for the same stage it is different for nearly every temperature interval studied. Since Bliss (1926) used only the prepupal stage of *Drosophila*, his results cannot be used as an index to the

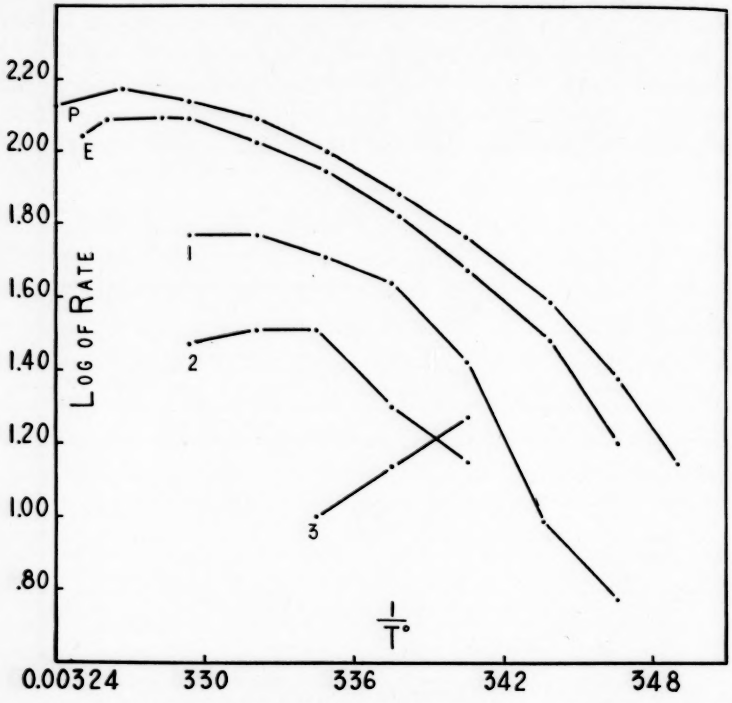


FIG. 9.—Abscissae, reciprocals of the absolute temperatures; ordinates, logarithms 1,000×the rates. *P*, pupal stage; *E*, egg; (1) first-instar larva; (2) second-instar larva; and (3) third-instar larva.

reactions of the other stages. Bonnier (1926) showed that in *Drosophila* there is an interrelationship between the stages of the life-cycle, so that conclusions cannot be reached on the basis of the reactions of only one stage. From these studies it appears that the temperature characteristics of growth cannot be used in an analysis of its nature.

(3) Krogh's formula as applied to growth and development: To express the relation between temperature and the rate of development, Krogh (1914) advanced the formula $V_{t+10°} = V_t + K_{10}$, where V_t is the velocity at one temperature and K_{10} is the increase in velocity for each 10° C. rise in temperature. It differs from the van't Hoff formula in that the constant is added, instead of being multiplied, to the rate at one temperature in order to obtain the rate at a higher temperature. This means that within certain limits usually described as the limits of normal development, an increase of 1° always produces the same acceleration in the rate of development. The time-temperature curve is, within these limits, a hyperbola, and the rate-temperature curve is a straight line which crosses the temperature axis near the threshold or zero temperature of development. This is demonstrated by the works of Sanderson and Peairs (1913), Krogh (1914), Peairs (1914), Glenn (1922), Lathrop (1923), and Shelford (1927). Krogh found that at the lower temperatures the observed results deviate from the straight line, producing an actual zero below that calculated from the curve. Chapman (1925) stated that this observed zero cannot be used in calculating the effective temperatures, since it lies off the hyperbola curve. Shelford (1927) concluded that, since the calculated is not the actual zero, it cannot be used in calculating effective temperatures. Krogh (1914) also demonstrated that the straight-line relation holds only for the temperatures below the optimum of development. Above that temperature the rate is retarded. Glenn (1922) assumed that, for the codling moth, the rate above the optimum decreases to the same extent that it increases as the temperature is increased up to the optimum. After making corrections based on this assumption, he obtained results which followed the straight-line rate curve. However, Glenn used data obtained under field conditions where the temperature is variable, and the method may not apply to results obtained under controlled constant temperature conditions. Blackman (1905) and Kanitz (1915) correlated the decrease in rate at higher temperatures with the reduction in the amount of active enzymes present in the tissues; while Gray (1923) associated it with a lack of oxygen in the tissues. Because the observed results deviate from the straight line at the upper and lower extremities, Krafka (1921) assumed that the

relation is not in reality a linear curve but a modified exponential curve. Friend (1927), working on the birch-leaf skeletonizer *Bucculatrix canadensisella*, and Cook (1927), working on the cutworm *Porosagrotis orthogonia*, obtained sigmoid curves when the rates were plotted against the temperature. These investigators do not present sufficient data to determine whether the curves obtained are straight or sigmoid between the upper and lower extremities where the results usually deviate from the straight line.

Peairs (1914), Glenn (1922), Shelford (1927), Korschelt (1924), and others have calculated thermal constants of development from the time-temperature curves by multiplying the number of effective degrees by the number of days required for the development of any stage or stages in the life-cycle at a given temperature, the effective temperatures being calculated by subtracting the threshold temperature for the stage from the temperature at which the animals were kept. The result is the number of day-degrees required for the development of the stage in question. Shelford (1927) made the analysis more accurate by calculating "developmental units." A developmental unit he defined as "the difference in amount of development produced in one hour by a difference of one degree of mean medial variable temperature (other conditions being average), as shown by the difference in time required to complete the stage." These investigators obtained thermal constants of development within the range of the straight line. Friend (1927) stated that, in his experiments, at no two consecutive temperatures were the number of degree-hours equal, or approximately equal, and that the curves show that no thermal constants exist in the development of the larva of the birch-leaf skeletonizer under experimental conditions.

The results obtained for the development of the Japanese beetle were analyzed according to the method of Krogh. The rate curves in Figures 1–5 and 8 show that for the egg and pupa the increase in rate is proportional to the increase in temperature. The rates of the larval stages, being modified by the inception of the resting period and by the food of the larva, do not follow the straight line as well as do the other stages. The first instar has a rate curve which is decidedly sigmoid (Fig. 2), but an examination of the results show that at 17.5° C. the larvae remained in this stage longer than had

been expected from the rates at other temperatures, on account of the inception of the resting period. This is also proven by the fact that the second instar at this temperature was correspondingly shorter, as shown in Figures 3 and 6. The rate for the first instar at 22.5° C. was accelerated more than was expected on the basis of the rates at other temperatures. More recent experiments seem to indicate that the rate calculated from the curve is more accurate than the observed rate at this temperature. The third instar, being

TABLE V

DAY-DEGREES REQUIRED FOR DEVELOPMENT OF EACH STAGE

Temperature (C.)	Egg	First Instar	Second Instar	Third Instar	Pupa
13°	.....	.....	.....	.....	35.24
15°	123.0	240.0	315.7	.....	104.0
17.5°	146.7	454.4	320.0	.....	126.5
20°	146.3	245.1	456.9	340.6	128.2
22.5°	141.5	201.0	448.2	676.8	129.0
25°	133.2	221.9	351.9	1141.9	122.0
27.5°	136.3	238.0	430.9	.....	120.0
30°	135.0	278.8	551.1	.....	126.8
31°	144.0	.....	.....	.....	.....
32.5°	.....	.....	.....	.....	133.0
35°	.....	.....	.....	.....	168.7

dependent on the lengths of the other larval stages, shows an inverse relation (Figs. 4 and 8). Food is a factor in the development of the larval stages which cannot be very accurately controlled because of differences which probably exist in the composition of the plant mold and in the quantity of food eaten by the larvae. All the curves show a decrease in rate above a certain (the optimum) temperature. For the pupa the rate is higher at 13° C. than that calculated from the straight line. The number of day-degrees shown in Table V is approximately constant between 15° and 30° C. for the egg, first instar (except at 17.5° C.), and pupal stages. In making the calculations, the temperature at which the rate curve crosses the temperature axis was used as the threshold temperature. The complete life-cycle, as shown in Table VI, does not have a constant number of day-degrees at the different temperatures, but the number increases with the temperature.

It may, therefore, be said that the formula of Krogh applies to the development of the egg and pupal stages but not to all the larval stages of the Japanese beetle. The method is of practical value, where it applies, because if the rates of development are known at several temperatures, it is possible to predict with accuracy the rates of those stages at other temperatures, within the limits of the straight line. The method explains the actual relations existing between temperature and the rate of development, without attempting to analyze the processes involved. These investigations seem to

TABLE VI

DAY-DEGREES REQUIRED FOR THE COMPLETE LIFE-CYCLE

Stage	20° C.	22.5° C.	25° C.
Egg	146.3	141.5	133.2
First instar	245.1	201.0	221.9
Second instar	456.9	448.2	351.9
Third instar	340.6	676.8	1141.9
Pupa	128.2	129.0	122.0
Total	1,317.1	1,596.5	1,970.9

indicate that, at the present time, growth and development cannot be analyzed in terms of the laws of chemical reactions. Bayliss (1924) and Heilbrunn (1925) point out the dissimilarity between the reactions taking place in a homogeneous solution and a heterogeneous system (such as protoplasm). Bayliss mentions several physical processes, such as diffusion and adsorption, which take place simultaneously with the chemical processes, and states that the velocity of the process as a whole is conditioned by the factor which takes place at the slowest rate, concluding that in many cases it is diffusion.

The effects of alternating as compared with constant temperatures.—The generalization has been frequently made that alternating or varying temperatures have a stimulating effect on development, as compared with their mean constant temperatures. Peairs (1914) advanced the tentative law that the effective temperatures calculated for variable are higher than for constant temperatures. If this is true, the rate of development would be accelerated. Peairs stated that he had no evidence to support the view. Headlee (1914), work-

ing with the insect *Toxoptera graminum*, found that a constant temperature was more effective in development and considerably shortened the growth stages. Chapman (1925) included some of Parker's unpublished data on *Melanoplus atlanis* which seem to support the idea that varying temperatures have an accelerating effect. He stated, "It is evident that the constant calculated from the time and effective temperature has a lower value in the case of varying temperatures than in the case of constant temperatures and that this value is lowest when a temperature below the minimum effective temperature is involved." His constants are only slightly less for varying than for constant temperature, except when a temperature below the minimum of development is involved. Shelford (1927) found in his codling-moth investigations that variable temperatures accelerate the development of the pupa 7 per cent, of the egg 7 per cent, and of the larva 8 per cent, as compared with constant temperatures. However, he also found that "in some exceptional experiments with small numbers of individuals, when the temperature rose suddenly and dropped again within a few hours, the velocity seemed to be decreased as compared with that for the corresponding constant temperatures." His experiments were conducted under the varying conditions found in the field and were not verified by carefully controlled experiments. To compare the effects of alternating with constant temperatures, Cook (1927) conducted a series of experiments on the first-instar larva of the cutworm *Porosagrotis orthogonia* and obtained accelerating effects. He conducted all his experiments using 8° C. as the minimum, alternated for different daily periods with various high temperatures. He did not perform a control experiment at 8° C. but calculated the rate of development from his rate curve at the higher temperatures. This curve, which he assumed to be sigmoid, shows that 8° C. is near the threshold temperature. Consequently, his results cannot be used to generalize in respect to the effects of alternating temperatures; and they are not very conclusive, since he used only eleven individuals for each experiment.

The experiments performed on the Japanese beetle show that the effects produced by alternating temperatures depend on the temperatures involved. (1) If one of the temperatures is above the

optimum of development and the other is between the threshold and the optimum, the rate is retarded. This may be explained by assuming that, while the organisms are exposed to temperatures above the optimum, the development is retarded to such a degree that the total developmental rate is lower than at the corresponding constant temperature. Glenn (1922) assumed that the rate is increased regularly up to an optimum temperature and then is decreased at the same rate. He, therefore, deducted twice the number of day-degrees accumulated above the optimum to correct for their retardation. It is interesting to note that if this correction is applied to these results obtained on the Japanese beetle, the corrected value is equal to that obtained at the mean constant temperature. For example, the number of day-degrees required for the development of the pupa at the alternating temperatures of 25° and 35° C. was 154.5. This number is changed to 122.3 by applying Glenn's correction. Table V shows that this value is the same as those obtained for the constant temperatures between 15° and 30° C. (2) If both the temperatures alternated are between the threshold and the optimum of development, neither an acceleration nor a retardation of the rate is produced. (3) If one of the temperatures involved is below the threshold of development and the other is between the threshold and the optimum, the rate is accelerated. However, if the threshold temperature is used as the minimum in the calculation of the mean constant temperature, the rate falls on the straight line. For example, for the egg the rate $\left(\frac{1}{\text{time}}\right)$ at the alternating temperature of 10° and 20° C. was 0.032 (Fig. 1). The mean constant temperature, calculated between 13.2° (the threshold) and 20° C., is 17.1° C.; and the rate at that temperature, calculated from the rate curve, is 0.030. The rate for the pupa at 10° and 20° C. was 0.035 (Fig. 5), and the rate at 16.8° C. (the mean between the threshold and 20° C.) is 0.035. Just why this condition exists is not evident, since it is assumed that, in this case, development can take place only while the organisms are at 20° C.

These results may help in explaining the apparently contradictory results obtained by various investigators. Most investigations are made under field conditions where the temperature is vari-

able and where it frequently goes below the thresholds of insect development. This may account for the opinion that alternating or varying temperatures accelerate insect development. On the other hand, results obtained on one species may not apply to another, or those obtained on one stage of the life-cycle may not apply to the other stages of the same species. Harrington (1923), working on the use of alternating temperatures on the germination of seeds, found that the seeds of some plants germinate as rapidly at favorable constant temperatures as with any alternation of temperatures, while other seeds require an alternation of temperatures for best germination. A similar condition may be found to exist in the responses of various animals to varying, as compared with constant, factors of the environment.

SUMMARY

1. Different stages of the life-cycle of the Japanese beetle are not affected to the same degree by temperature. The threshold, optimum, and maximum temperatures are different for each stage. The egg and pupal stages are very regular in their temperature responses; but the larval stages are modified by other factors, such as food and the inception of a resting stage. The presence of wheat in the food seems to hasten the development of those stages not affected by the resting stage; but the total larval period is not affected, since the resting stage is correspondingly longer.

2. The temperature coefficients (Q_{10} values) are not constant for any stage. They diminish regularly as the temperature is increased for each stage, except the third-instar larva, which is modified by the behavior of the other larval stages.

3. The temperature characteristics (μ values) as derived from the Arrhenius formula are not constant for any stage. They diminish regularly as the temperature is increased, except for the third-instar larval stage.

4. Krogh's formula, that the rate of development is increased in direct proportion to the increase in temperature (within certain limits), has been found to apply to the egg and pupal stages. The number of day-degrees required for the complete life-cycle is not constant for different temperatures but increases with the temperature.

5. The effects of alternating temperatures, as compared with constant temperatures representing their mean values, depend upon the temperatures involved. (*a*) If one of the temperatures is above the optimum of development and the other is between the threshold and the optimum, development seems to be retarded. (*b*) If both of the temperatures are between the threshold and the optimum, neither an acceleration nor a retardation of rate is produced. (*c*) If one of the temperatures is below the threshold and the other is between the threshold and the optimum, the development seems to be accelerated.

LITERATURE CITED

ARRHENIUS, S. 1915. Quantitative laws in biological chemistry. London. P. 49.

BAYLISS, W. M. 1924. Principles of general physiology. London. P. 43.

BLACKMAN, F. 1905. Optima and limiting factors. Ann. Bot., XIX, 281.

BLISS, C. I. 1926. Temperature characteristics for prepupal development in *Drosophila melanogaster*. Jour. Gen. Physiol., IX, 467.

BONNIER, G. 1926. Temperature and the time of development of the two sexes in *Drosophila*. Brit. Jour. Exp. Biol., IV, 186.

BROWN, L. A. 1926. Temperature characteristics for the duration of an instar in *Cladocerans*. Jour. Gen. Physiol., IX, 467.

CHAPMAN, R. N. 1925. Animal ecology with especial reference to insects. Minneapolis.

COOK, WILLIAM C. 1927. Some effects of alternating temperatures on the growth and metabolism of cutworm larvae. Jour. Econ. Ent., XX, 769.

CROZIER, W. J. 1924. Critical thermal increments for the locomotion of a diplopod. Jour. Gen. Physiol., VII, 123.

———. 1924. On biological oxidations as a function of temperature. *Ibid.*, VII, 189.

FRIEND, R. 1927. The biology of the birch-leaf skeletonizer, *Buccalatrix canadensisella*. Conn. Agr. Exp. Sta. Bull. 288.

GLENN, P. A. 1922. Codling-moth investigations. Ill. Nat. Hist. Survey Bull., XIV, 119.

GRAY, J. 1923. The mechanism of ciliary movement. III. The effects of temperature. Proc. Roy. Soc. London, Vol. XCV B.

HARRINGTON, GEO. T. 1923. Use of alternating temperatures in the germination of seeds. Jour. Agr. Res., XXIII, 295.

HEADLEE, T. J. 1914. Effects of temperature and moisture on the rate of insect metabolism. Jour. Econ. Ent., VII, 413.

HEILBRUNN, L. V. 1925. The use of temperature coefficients in the interpretation of biological processes. Science, LXII, 268.

HERTWIG, O. 1898. Über den Einfluss der Temperatur auf die Entwicklung von *Rana fusca* und *Rana esculenta*. Arch. Mikr. Anat., XXI, 319.

KANITZ, A. 1915. Temperatur und Lebensvörgange. Berlin.

KORSCHELT, E. 1924. Bearbeitung einheimischer Tiere. Leipzig. P. 730.

KRAFKA, J. 1920. Effects of temperature upon facet numbers in the bar-eyed mutant of *Drosophila*. Jour. Gen. Physiol., II, 445.

———. 1921. The physiological zero: An explanation of the departure from the linear graph of reaction rate values at the lower temperatures. *Ibid.*, III, 659.

KROGH, A. 1914. On the influence of temperature on the rate of embryonic development. Zs. Allg. Physiol., XVI, 163.

LATHROP, F. 1923. Influence of temperature and evaporation upon the development of *Aphis pomi*. Jour. Agri. Res., XXIII, 969.

LOEB, J. 1908. Ueber den Temperaturkoeffizienten für die Lebensdauer kaltblütiger Thiere und über die Ursache des natüralichen Todes. Arch. f. d. ges. Physiol., CXXIV, 411.

LOEB, J., and NORTHROP, J. H. 1917. On the influence of food and temperature upon the duration of life. Jour. Biol. Chem., XXXII, 103.

LOEB, J., and WASTENEYS, H. 1911. Sind die Oxydations-vorgänge die unabliängige variable in den Lebenserscheinungen? Biochem. Zeitsch., XXXV, 347.

PEAIRS, L. M. 1914. The relation of temperature to insect development. Jour. Econ. Ent., VII, 174.

ROGERS, CHARLES. 1911. Studies upon the temperature coefficients of the rate of heart beat in certain living animals. Amer. Jour. Physiol., XXVIII, 81.

SANDERSON, E. D. 1910. The relation of temperature to the growth of insects. Jour. Econ. Ent., III, 113.

SANDERSON, E. D. and PEAIRS, L. M. 1913. The relation of temperature to insect life. N.H. College Agr. Exp. Sta. Tech. Bull. 7.

SHELFORD, V. E. 1927. An experimental investigation of the relation of the codling moth to weather and climate. Ill. Nat. Hist. Sur. Bull., XVI, 315.

SYNDER, C. D. 1911. On the meaning of variation in the magnitude of temperature coefficients of physiological processes. Amer. Jour. Physiol., XXVIII, 167.

VAN'T HOFF, 1884. Études de dynamique chemique. Amsterdam. P. 116.

A SOURCE OF ERROR IN METABOLIC GAS-EXCHANGE MEASUREMENTS

(One figure)

G. EDWARD SPENCER
Research Laboratory, Bureau of Entomology, Moorestown, New Jersey

THE present method of studying basal metabolism in insects and some other animals by means of a Krogh manometer requires the use of apparatus for the maintenance of constant temperature. The heating-units for this apparatus usually consist of one or more light bulbs controlled by a thermostat. That these light bulbs might affect the results through action of radiant energy has hitherto been entirely overlooked by investigators.

During gas-exchange measurements, in studies on cocoons of *Tiphia vernalis* Rohwer, a hymenopterous parasite of the Japanese beetle, an unusual condition, indicated by the readings, occurred in the manometer nearest the light bulb. It was noted that this effect invariably took place when the light was turned on, and only then, and that its intensity varied inversely with the distance between the light bulb and the manometer bulb. When a thermometer bulb was placed in the water bath between the light bulb and that of the manometer, although the reversed reading of the manometer was marked, no temperature change was indicated by the thermometer.

To determine whether this reversed reading was due only to gas expansion caused by the absorption of radiant energy by the air and other gases in the manometer bulbs, observations were made in which the bulbs contained 2 cubic centimeters of potassium hydroxide solution, or a few drops of water only. Although there was a slight effect, it was very small when compared to that observed when an insect was present. It was found that when the light bulb was covered with black carbon paper or with light metal foil, there was little or no effect on the normal readings of the manometers. On the other hand, when the manometer bulbs were covered with light metal foil, a slight effect was noted.

The light bulb was next covered with lead foil, in which an aperture of approximately 1 square centimeter was so cut that the light rays fell directly upon the manometer bulb, which was placed exactly $2\frac{1}{2}$ centimeters from the surface of the light bulb. The effect of the rays on the insect in the manometer bulb now yielded definite and comparable readings.

When two plates of ground glass were placed between the light bulb and the manometer bulb, the reversed reading was reduced approximately by one-half, which was another indication[1] that it was the absorption by the insect of radiant energy from the light bulb that affected the readings. When stage-lighting filters of different colors were substituted for ground glass, it was found that while red passed about half of the rays that caused the reversed readings, the other colors absorbed almost all of them.

Several improvements were then made in the apparatus. The light bulb was covered with two thicknesses of lead foil. A small sheet of heavy tin plate, with an aperture of exactly 1 square centimeter, was bent to conform to the surface of the bulb and was placed under the lead foil. The foil was cut to expose the aperture, which was fixed in a direct line between the filament of the lamp and that part of the manometer bulb in which the insect was suspended. During the entire course of the experiments the water in the constant temperature bath was continually agitated by an electric-motor-driven propeller.

Tests were made in which the full light of the lamp was allowed to fall upon the manometer bulb, and in which ground glass and red (G 24), green (G 401–CZ), blue (G 54), and violet (G 53C) filters made by the Corning Glass Company were placed between the source of light and the manometer bulb. The readings in each test were compared with those of tests in which no light was used. The latter were taken as the standard of measurement. Figure 1 shows that the greatest effect takes place during the first minute and that the maximum effect has occurred by the end of the second minute. Two-minute periods were therefore taken as a standard. Each value recorded in this paper is an average of three readings.

[1] L. Bell, *Illumination*, Table V ("Loss of Light in Passing through Closed Shades"); L. S. Mark, *Mechanical Engineers' Handbook* (McGraw, Hill, 1916), p. 1377.

In order to determine the respiratory quotient, a 2 per cent solution of potassium hydroxide was used in the manometer bulbs when one of the two series of readings was being made, to absorb the carbon dioxide. In the presence of this solution the results were relatively the same as when the bulbs contained a few drops of water only. The same individual insects were used with and without potassium hydroxide.

The absorption of radiant energy so affects the insect forms which were studied that a definite change occurs in the volume of

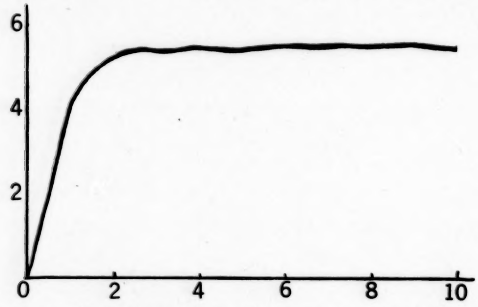


FIG. 1.—Effects of radiation absorbed in 10 minutes. Ordinates are millimeters of manometer tube; abscissas are minutes.

the gas in the manometer. Table I shows that this effect was similar on different types and stages of insects. The readings, which are given in longitudinal millimeters of the manometer tubes, are all *reversed* readings, unless preceded by a minus sign. That is, the effect of the radiant energy upon the insect results in movement of the oil column *away* from the bulb which contains the insect, instead of *toward* it, as occurs in normal respiration.

It is the usual practice to suspend insects in cotton gauze or wire-gauze baskets in the manometer bulb. In one case, a larva of the Japanese beetle was tested while suspended by a cotton thread, after one had been tested in a copper-gauze basket. The results differed widely in the two tests. The effect of the radiant energy on the copper-gauze basket only was then tried and was found to be greater than when the insect too was present.[1]

[1] This suggests a possible method for the quantitative measurement of the absorption of gases by metals. When platinum wire was substituted for the copper gauze, a similar result was observed.

TABLE I

EFFECTS OF RADIANT ENERGY ON INSECTS AND CONTAINERS IN THE KROGH MANOMETER
(Water Bath at 25° C.)

	With Copper-Gauze Basket				Without Copper-Gauze Basket				
	Tiphia Cocoon	Japanese-Beetle Larva	Grasshopper Eggs	Basket Only	Mantis Eggs	Io Moth Pupa	Japanese-Beetle Larva	Japanese-Beetle Larva in White Gauze	Empty Manometer Bulb
Without KOH									
Full light	4.18	3.90	2.13	3.93	2.80	1.63	1.70	1.56	0.72
Red screen	1.73	2.23	1.23	2.00	2.10	1.06	0.53	0.96	0.00
Green screen	0.05	−0.01	0.23	0.00	0.00	−0.46	−0.03	−0.16	0.00
Blue screen	0.24	0.03	0.26	0.00	−0.20	−0.23	−0.06	−0.10	0.00
Violet screen	0.00	−0.33	0.13	0.00	−0.20	−0.13	0.13	−0.40	0.00
Control—no light	0.10	−0.50	−0.17	0.00	−0.15	−0.56	−0.10	−0.56	0.05
With KOH									
Full light	4.60	2.90	1.80	4.90	3.67	0.93	1.40	1.26	0.86
Red screen	2.45	1.20	1.06	2.66	2.00	−0.70	0.76	0.23	−0.05
Green screen	−0.05	−0.66	0.00	0.00	−0.26	−1.53	−0.36	−0.96	0.00
Blue screen	−0.13	−0.56	0.20	0.00	−0.26	−1.80	−0.36	−0.93	0.00
Violet screen	−0.18	−0.43	−0.06	0.00	−0.30	−1.60	−0.36	−1.06	0.00
Control—no light	−0.21	−1.13	−0.20	0.00	−0.26	−1.90	−0.40	−1.03	0.00

CONCLUSIONS

In the measurement of gas exchange of insects by means of a Krogh manometer, absorption of radiant energy of long wave-length from the light bulb in the heating unit affects the readings.

The action of radiant energy upon copper gauze alone in the manometer bulb causes a reversed reading greater than that when an insect also is present.

The light bulbs ordinarily used for controlling the temperature in gas-exchange measurements are the cause of errors. Data hitherto obtained with this type of apparatus must therefore be corrected for these errors.

ULTRA-VIOLET RADIATION AND DIVISION IN PARAMECIUM CAUDATUM

(Eight Figures)

MARIE A. HINRICHS
Physiological Laboratory, University of Chicago

MANY agents have been used to modify the division rate in *Paramecium caudatum*, and both inhibition and stimulation of rate have been reported.[1] In some instances the same agent produced either inhibition or stimulation, the effect depending on the dosage of the agent employed. Bovie and Hughes (1918) report the modification of the division rate of *P. caudatum* by means of ultra-violet radiation as follows: the duration of inhibition increases with increasing exposure, and an inhibition may be followed by an acceleration. They attribute the accelerative effects following short periods of inhibition to the formation of a "toxic photoproduct which is gradually removed from the cell," and which "acts as a stimulant to cell division when the amount becomes very small. Many toxic substances are known which in small doses have a stimulative action."

The experiments to be reported in this paper confirm the results of Bovie and Hughes in that both stimulation and inhibition of division rate have been found, and the effect depends on the dosage of radiation used. Similar results have been reported in the modification of the division rate of other cells, and in the action of radiation on enzyme activity. In this connection see Cattley, 1909; Packard, 1916 and 1924; and Richards 1914*a*, 1914*b*, and 1922. Further indications of a two-directional effect are seen in the experiments with alkaloids and with ultra-violet radiation previously reported (Hinrichs, 1924 and 1927) and in unpublished data (Hinrichs) on alkaloid modification of development in *Arbacia*.

Acknowledgment.—I wish here to express my gratitude to Dr. R. S. Lillie for his helpful suggestions during the study of this problem.

[1] See Calkins, 1926; De Garis, 1927; Packard, 1924; and Richards, 1914*a*, for a discussion of the literature on the division rate in *Paramecium*.

METHOD

In these experiments, *P. caudatum* was exposed individually, in small groups, or *en masse*, to radiation from a Cooper-Hewitt quartz mercury-vapor lamp running at 110 volts, D.C., and about 4 amperes. Exposures were made in open dishes at 26.5–45 cm. for periods of 5 to 30 seconds, and at 56 cm. for 80 seconds. Distance was measured from the center of the arc to the table. Animals were exposed during the interdivisional period and at various stages during the division cycle. Cultures were kept well fed on a wheat infusion; and fresh medium was made every other day, or whenever the division rate in the culture slowed down.

RESULTS

The following studies have been made, and the results of each type of experiment will be discussed separately: modification of the rate of division; effect of exposure on the duration of each stage in the division cycle; prevention of fission; production of "permanent" pairs, conditions of production, and percentage of survival of such pairs under various conditions; relative frequency of division of conjoined anterior and posterior daughter cells; relative susceptibility to lethal doses of ultra-violet radiation of the various components of chains of two, three, and four cells; behavior of such chains.

Modification of the rate of division in Paramecium caudatum, following exposure during the interdivisional stage.—In these experiments, from 10 to 20 single *Paramecia* were isolated on depression slides in equal volumes of 1-day old medium (1 animal to 4 drops) and kept in a moist chamber for 24 hours, at the end of which time the division products were counted for each individual. The total offspring of individuals with a given division rate was then used as control, while the total offspring of an individual with the same division rate was used as test. Controls were kept on depression slides in a moist chamber, singly, or in groups of 2, 4, 5, or 8, per 4, 5, or 10 drops of medium. (See Table I, cols. 3 and 4.) The animals to be tested were placed on depression slides in the same proportions of animals to medium, and radiated.

The total number of offspring was counted daily for radiated and non-radiated animals, in each case, and the rate of division calcu-

TABLE I

Effect of Ultra-violet Radiation on Division Rate in *Paramecium caudatum*

A. Stimulation

Dist. Exposed in Cm.	Seconds Exposed	No. of Animals	No. of Drops	Exposed Singly, Paired, or All Together	Total No. of Animals Exposed	Rate of Division (No. of Divisions per Animal)						Final No. of Animals	
						Control			Test				
						1st Day	2d Day	3d Day	1st Day	2d Day	3d Day	Control	Test
26.5	5	4	5	Together	4	0	1	1+	0	2	3	9	32
	10	4	5	Together	4	0	1	1+	0	2	2+	9	23
	15	4	5	Together	4	0	1	1+	0	2	3−	9	29
	25	2	5	Together	2	0	2	3−	0	2	3	15	16
	30	2	5	Together	2	0	2	3−	0	2	3	15	16
37.0	6	4	5	Together	4	2−	3−	4+	2−	3−	5−	85	107
	10	5	5	Together	5	4+	5+	6+	4−	5+	6+	341	444
38.5	5	4	4	Together	4	0	2	4+	0	3−	6−	65	194
	15	1	4	Singly	4	0	1	2	0	2	5+	16	129
	15	8	4	Together	8	0	2	3+	0	1−	4−	73	123
	15	4	4	Together	4	0	1	3+	0	2	4−	43	60
45.0	1	2	5	Together	2	2	3−	3+	2	3	4+	23	34
	3	2	5	Together	2	2	3−	3+	3−	4	5	23	64
	5	2	5	Together	2	2	3−	3+	2	4	4+	23	37
	10	2	5	Together	2	2	3−	3+	2−	4−	5−	23	55
	20	2	5	Together	2	2	3+	4−	2	3	4−	28	31
	20	2	5	Together	2	2	4−	4−	1	3	4	29	32
	20	1	5	Singly	2	2−	3−	3+	1	3−	4−	18	30
Total number exposed					59								

TABLE I—*Continued*

B. Inhibition

Dist. Exposed in Cm.	Seconds Exposed	No. of Animals	No. of Drops	Exposed Singly, Paired, or All Together	Total No. of Animals Exposed	Rate of Division (No. of Divisions per Animal)						Final No. of Animals	
						Control			Test				
						1st Day	2d Day	3d Day	1st Day	2d Day	3d Day	Control	Test
26.5	5	2	5	Paired	4	o	1+	3−	o	1+	3−	28	26
	10	8	5	Together	16	o	1+	3+	o	1+	3	183	128
	15	8	5	Together	16	o	1+	3+	o	o+	2+	160	65
	15	4	5	Together	4	o	2	4−	o	1	3+	60	38
	25	1	5	Singly	4	o	1	3	o	1−	3	*512	*432
	25	1	5	Singly	1	o	3	4+	o	1	2	17	4
37.0	5	1	3	Singly	5	o	1+	3	o	o+	2+	†92	†70
	7	3	5	Together	3	1	2−	4−	2−	2	3+	39	33
	15	4	5	Together	4	2−	3−	4+	o+	1	3−	85	25
	23	1	10	Singly	3	2	4−	5+	o	2−	3+	135	30
	23	1	5	Singly	3	2−	3−	5+	1−	2	4−	97	40
	23	1	5	Singly	4	1+	3	5+	o	1+	3−	123	24
	30	5	5	Together	5	4+	5+	6+	3−	4−	5−	341	155
38.5	15	3	4	Together	3	o	2+	5−	o	1	4+	80	50
	20	4	4	Together	4	o	2	4+	o	1−	3+	65	39
	30	1	4	Singly	4	o	2	4+	o	o	3+	65	43
45.0	15	1	5	Singly	1	o	3	4	o	1	2	†16	†8
56.0	80	5	5	Together	5	4+	5+	6+	3−	4+	5+	341	179
Total number exposed					84								

* Final count for 5 days. † Final count for 4 days.

lated each day for 3 successive days, and in a few cases, for 4 or 5 days. These calculations are recorded in Table I under the heading of "Rate of Division." The last column of the table represents the final number of animals found on the slide at the end of each experiment. A comparison of these two figures, for control and test, in each experiment, forms the basis for the calculated percentage of gain or loss in rate of division, as listed in Table II under the headings, "Percentage of Stimulation" and "Percentage of Depression," respectively.

The degree of depression, as calculated on the basis of total offspring of radiated and non-radiated animals, ranged from 7 to 82 per cent below normal. In the majority of the experiments in which depression of division rate occurred, exposures were made for 5 to 30 seconds at a distance of 16.5 to 38.5 cm. from the lamp. One experiment, with an exposure at 45 cm. for 15 seconds, and one at 56 cm. for 80 seconds also produced a depression in rate of division and in the total offspring produced (see Table II).

Table II shows the relation of dosage to the frequency of stimulation and depression. It will be seen from a study of column 3 that the largest amount of stimulation is obtained by short exposures made at any distance from the lamp. The percentage of depression, as recorded in column 7, increases with the length of the period of exposure. Columns 5 and 9 represent the total number of experiments in which stimulation and depression occurred, respectively. It will be seen that exactly one-half of the experiments resulted in depression.

On the other hand, 18 of the 36 experiments showed an increase of from 7 to 706 per cent over the normal total offspring production of the non-radiated controls. In these experiments, exposures were made for 5 to 30 seconds at 26.5 cm. from the lamp, and for 6 to 20 seconds at 37 to 45 cm. It was found that the number of cases where stimulation occurred was greater when exposures were made at 38.5 cm., or more, away from the lamp. Seventy-seven and eight-tenths per cent of the exposures made at or beyond 45 cm. produced stimulation, whereas only 44.8 per cent of the exposures made at closer range showed an increase in division rate and total offspring produced. Depression of the rate of division may be seen during the

first days following radiation, but stimulation usually does not appear until within the second 24 hours. Markovits (1922) was able to produce an inhibition of the rate of the first division in *Paramecium* by means of mesothorium. The second division showed an

TABLE II

THE RELATION OF DOSAGE TO THE FREQUENCY OF STIMULATION AND DEPRESSION

Dist. in Cm.	Time Exposed in Sec.	Stimulation				Depression			
		Percentage of Stim. in Each Expt.	No. of Animals in Expt.	No. of Expts.	Percentage of Cases Stim.	Percentage Depr. in Each Expt.	No. of Animals in Expt.	No. of Expts.	Percentage of Cases Depr.
	5	256	4			7	4		
	10	156	4			30	16		
	15	222	4			59	16		
26.5	15	.....	.....	5	45.5	37	4	6	54.5
	25	7	2			15	4		
	25	.....	.....			76	1		
	30	7	2			.....	.....		
	5	.....	.....			24	5		
	6	26	4			.....	.....		
	7	.....	.....			15	3		
	10	30	5			.....	.....		
37.0	15	.....	.....	2	22.2	71	4	7	77.8
	23	.....	.....			78	3		
	23	.....	.....			59	3		
	23	.....	.....			82	4		
	30	.....	.....			56	5		
	5	198	4			.....	.....		
	15	706	4			.....	.....		
38.5	15	68	8	4	57.1	.....	.....	3	42.9
	15	40	4			37	3		
	20	.....	.....			40	4		
	30	.....	.....			34	4		
	1	48	2			.....	.....		
	3	178	2			.....	.....		
	5	37	2			.....	.....		
45.0	10	139	2	7	87.5	.....	.....	1	12.5
	15	.....	.....			50	1		
	20	11	2			.....	.....		
	20	10	2			.....	.....		
	20	67	2			.....	.....		
56.0	80	.....	.....	0	0.0	48	5	1	100.0

increase in rate. The author suggests that a short exposure stimulates nuclear division, but, since there is a discrepancy between this new rate of division of the nuclear apparatus and that of the rest of the cell, division of the cell is slowed. When, however, a physiological balance is reached, cell division goes on at an increased rate.

Modification of division rate following exposure during the various stages of the division cycle.—In these experiments, the division cycle was arbitrarily separated into three stages: Stage I, in which an equatorial thickening appears; Stage II, in which a slight constriction occurs at the equator; and Stage III, in which the two daughter cells are well formed but have not yet separated. A rather wide protoplasmic connection still persists between the two cells.

TABLE III

THE EFFECT OF EXPOSURES MADE DURING VARIOUS STAGES OF DIVISION

	Time in Sec.	Dist. in Cm.	Time after Radiation, in Days	Total No. of Offspring*			
				Non-dividing	Stage I	Stage II	Stage III
A	0 (controls)	.....	1	14	24	32	26
			2	27	47	47	47
			3	49	110	110	72
	6	37.5	1	12	18	18	26
			2	40	53	59	66
			3	88	111	125	120
	18	37.5	1	9	9	9	8
			2	25	24	25	19
			3	36	41	46	33
	80	56.0	1	8	10	10	7
			2	19	22	23	18
			3	40	52	53	34
B	0 (controls)	.....	1	13	23	19	23
			2	82	29	34	41
	6	56.0	1	15	19	21	19
			2	23	38	39	44
	24	56.0	1	17	19	21	18
			2	21	32	32	31

* Five animals in each stage of division and in the non-dividing state were exposed in each experiment. A similar set of five non-radiated animals was used as controls.

In these experiments, non-dividing animals, and animals in each stage of division, were kept on depression slides in the proportion of 5 animals to 5 drops of medium. A full series of non-radiated, non-dividing and dividing animals was kept in the same way to serve as controls. Table III shows the results of a number of such experiments. Exposures were made during the non-dividing period, and during Stages I–III as indicated above. The figures in Table III represent the total offspring at the end of each of three successive 24-hour periods in both non-radiated controls and in radiated animals.

It will be seen that stimulation occurs in all stages, following a 6-second exposure made at 37.5 cm. (A) and at 56 cm. (B); while depression of the division rate in all stages follows exposures of 18 seconds at 37.5 cm. and of 80 seconds at 56 cm. (A), and a slight depression at 56 cm. with a 24-second exposure (B). The total and average number of offspring per radiated individual were both less than for normal individuals in every case where inhibition was produced. The rate of division appears to decrease on the first day but to recover thereafter. However, the total number of offspring never quite equals that of the non-radiated control. Obviously, there may be either an increase or a decrease in the length of the division period following radiation.

In order to determine whether dividing animals were more susceptible to the inhibiting effect of radiation applied at any one stage of division than at any other, animals were again exposed at the various stages indicated above; and the length of time occupied in the completion of each stage was noted. A fourth stage was added, in which the two daughter cells are just ready to separate. Stage V represents complete separation of the cells. The results of such an experiment are given in Table IV. Column 1 of Table IV records the length of the periods of exposure. All exposures were made at a distance of 26.5 cm. Short exposures ranged from 5 to 10 seconds, and long exposures, from 27 to 32 seconds. The second column shows the number of animals exposed in each group, and the other columns represent the average time, per animal, consumed in passing from one stage in the division cycle to the next. The last column shows the average total time required to complete the entire division cycle.

As Table IV indicates, the length of each stage in the division cycle is increased when inhibiting doses of radiation are used. A notable increase in the length of the complete division period appears. Short exposures made during the early part of the division period shorten the length of time necessary for the completion of the division cycle. However, short exposures are not effective in producing an increase in the speed of division when radiation is applied later than the first stage, i.e., after the division process is well under way.

Prevention of division.—Since it is possible, with inhibiting doses

of radiation, to lengthen the division cycle and thus completely to slow down the division rate as indicated in the previous tables, it seemed probable that successive doses of radiation might completely eliminate the division cycle, thus maintaining the individual intact through a period corresponding to a great number of generations.

TABLE IV

INCREASE IN THE LENGTH OF THE DIVISION PERIOD AFTER RADIATION

TIME EXPOSED IN SEC.	NO. OF ANIMALS USED	AVERAGE TIME (IN MIN.) REQUIRED TO PASS THROUGH EACH STAGE				
		Stage I–II	Stage II–III	Stage III–IV	Stage IV–V	Total Time
0.....	3	12	6.3	9.7	7.0	35.0
5–10.....	4	4.8	7.3	9.8	8.0	29.0
27–32.....	3	10.3	22.3	20.0	16.7	69.3
27–32.....	2	36.5	18.0	30.0	perm.*	.....
0.....	3	.....	7.7	9.3	8.7	25.7
5–10.....	2	.....	4.5	13.0	10.5	28.0
27–32.....	4	.....	5.8	16.3	25.3	47.4
0.....	4	.....	.....	6.5	7.8	14.3
5–10.....	5	.....	.....	6.7	7.4	14.1
27–32.....	3	.....	.....	8.7	12.2	20.9
0.....	5	.....	.....	.....	4.3	4.3
5–10.....	2	.....	.....	.....	4.5	4.5
27–32.....	4	.....	.....	.....	15.3	15.3

*Perm.—In these animals, the two halves failed to separate and they remained as "permanent" pairs.

Accordingly the following experiment was made: Twenty non-dividing animals were selected from a mass of radiated animals and were kept singly on depression slides in a moist chamber. Successive exposures were made at intervals of from 12 to 24 hours. The initial exposure and successive exposures were made at a distance of 26 cm. for a period of 16 seconds. Ten non-radiated, non-dividing animals were similarly kept as controls. In each case, 1 animal was placed in 4 drops of medium.

At the end of the sixth exposure (i.e., after 3 days) the control was discontinued. The number of offspring from the ten controls totaled 271, or an average of 27.1 per individual. Of the 20 radiated animals, meanwhile, only a few had divided. The total offspring amounted to 28, or an average of 1.4 per individual.

In section A of Table V, the total offspring found prior to each exposure is recorded for radiated and non-radiated animals. Two exposures were made daily for 3 days, as previously stated; and the effects of such expsoures on the total number of offspring produced are recorded under A and B in the first six columns of the table. The last column of section A shows the average number of offspring per individual, i.e., the quotient obtained by dividing the last total recorded (col. 6) by the number of animals exposed (col. 1).

TABLE V

PREVENTION OF FISSION BY ULTRA-VIOLET RADIATION

A. Total Offspring Prior to Each Exposure

	First Day		Second Day		Third Day		Average No. per Individual
	A	B	A	B	A	B	
Radiated.....	20	20	24	26	25	28	1.4
Non-radiated....	10	21	33	50	98	271	27.4

B. Division and Survival of Undivided Radiated Animals*

Day of Death	No. Dead on Each Day	No. of Exposures Survived
1.....	1	2
2.....	2	4
3-5.....	1	6
4.....	3	7
5.....	1	8
6.....	2	9

* Number of radiated animals which divided—10 (8 once, 2 twice). Number of radiated animals which died without dividing—10.

Section B of Table V shows, in column 1, the day of death of each animal which failed to divide; the second column shows the number which died on each successive day; and the third column records the number of exposures survived by the animals referred to in the preceding column.

Ten of the 20 animals which were radiated divided as follows: 8 once, and 2 twice during the entire experiment, which lasted 6 days. The other 10 did not divide. They survived a varying number of exposures, as Table V, B, column 3 will indicate. The aver-

age total number of divisions per animal in the control lot at the end of the third day was close to 5; and that in the radiated lot less than 1.

Permanent pairs.—It was noted in some of the experiments in which exposures were made during early division stages that occasionally the two daughter cells of a radiated dividing animal failed to separate, and, after recovering from the initial slowing down of motion, swam around tandem-fashion, remaining paired and normally active for many days.

By radiating large numbers of *Paramecia* during early division stages for 16–20 seconds at 26 cm., it was found that a certain proportion remained paired after 24 hours. Of a total of 3,423 dividing animals thus exposed, only 1,163, or 33.9 per cent, were still paired on the following day. Table VI shows the results of a number of such experiments. Within the course of 20–30 hours after radiation, one or both of the members of such a tandem pair may again divide. If, then, a second exposure is made during the early stage of division of one or both of the daughter cells, as the case may be, a "permanent" three- or four-celled animal will result. See Figures 1–8 (p. 414), which show photographs of chains of cells which have been kept from separating by exposure to ultra-violet radiation, as described above. Cellular continuity does not inhibit the independent division of the members of the pair. However, the greater the degree of continuity (i.e., the wider the protoplasmic bridge connecting the two cells) the more nearly do the division periods of the two daughter cells coincide, and the greater the chance of production of four-celled individuals. If, however, one or the other of the two daughter cells divides long before the other cell is ready for division, usually only three cells are produced (Figs. 3–5) because the exposure which "fixes" the first division inhibits the onset of division in the second cell. In only a few instances has it been possible, under these conditions of unequal division rate of the two cells, to produce a chain of four cells by two successive exposures on the second day. Of the 1,163 "permanent" pairs, only 90, or 7.7 per cent, divided again.

In order to determine at which stage in the division cycle "permanent" pairs could be most easily produced, dividing animals were radiated at the various stages as previously indicated. The next day

the number of remaining pairs was counted and recorded in Table VII. It was found that radiation applied during Stage I (i.e., early in the beginning of the division cycle) produced by far the greater

TABLE VI

SURVIVAL IN PAIRED STATE FOLLOWING RADIATION OF DIVIDING CELLS

No. of Dividing Cells Exposed	Remained Paired Next Day		Divided Again (Producing "Threes" or "Fours")	
	No.	Percentage	No.	Percentage
268	183	68.2	22	12.0
430	58	13.6	6	10.3
455	115	25.2	1	.8
214	14	6.5	2	14.3
444	124	27.9	5	4.0
110	31	28.2	3	9.6
382	132	34.5	4	3.0
154	56	36.3	10	17.8
441	68	15.4	6	8.8
525	382	72.7	31	8.1
Totals: 3,423	1,163	33.9	90	7.7

TABLE VII

SURVIVAL AS PAIRS FOLLOWING EXPOSURE AT VARIOUS STAGES OF DIVISION

Non-dividing		Stage I		Stage II		Stage III	
No. Exposed	No. Surviving	No. Exposed	No. Surviving	No. Exposed	No. Surviving	No. Exposed	No. Surviving
11	8	14	8	43	13	3	0
20	18	30	23	43	17	10	3
18	3	15	1	44	6	34	0
17	1	13	1	31	0	7	1
17	9	21	14	54	9	13	0
Totals: 83	39	93	47	215	45	67	4
Percentage surviving...	46.9	.....	50.5	.....	20.9	.....	5.9

number of "permanent" pairs, and during Stage III, the least. Radiation during Stage III usually does nothing more than slow down the activities of the dividing animal, but the two daughter cells invariably separate eventually. Table VII shows records of a number of such observations.

Frequency of division in anterior and posterior daughter cells.—As previously indicated, either one of the two daughter cells of a tandem pair may divide before the other. A careful study was made of the relative frequency of prior division in each of these cells. At first it was thought that division occurred earlier in more cases in

TABLE VIII

FREQUENCY OF DIVISION IN ANTERIOR AND POSTERIOR CELLS OF CONJOINED PAIRS

DATE	THREE'S		FOUR'S		
	Ant. Div. First	Post. Div. First	Ant. Div. First	Post. Div. First	Simult.
Feb. 24	1				
Mar. 4					1
8			1	1	
10			2	2	
12	5	3			
14	2		1		
16		9	2		
18	6	2	7	4	2
23	10	13	4	4	2
27		2	1	1	
29	1	1	2	1	1
30				1	
31	4	5	1	1	
Apr. 1	2	3	2	3	
6					1
8	1	1			
16	1				1
May 6	1	1	1	5	
10		1	1	4	
16	5	1			1
Oct. 27	4	5	1	3	
Nov. 22	1	1		2	
Totals	44	48	26	32	9
Total no.	92		67		
Total no. of cell divisions	92		134		

the anterior than in the posterior cell; but after a larger number of pairs was studied, it was found that division occurred first about as often in the anterior as in the posterior cell. Table VIII shows the results of such a study. It will be seen that of a total of 159 pairs studied, 92, or 57.8 per cent, formed chains of three cells on second division, i.e., the difference between the two cells of a pair with regard to their relative rates of division was great. Of the 92 three's

thus produced, 47.9 per cent were due to division of the anterior cell, and 52.1 per cent to prior posterior division. In the formation of four's, 38.8 per cent resulted from the more rapid division in the anterior cell, 47.7 per cent resulted from the more rapid division in the posterior cell, and 13.5 per cent from simultaneous division of the two cells. There was a total of 226 second divisions (92 and 134); and of these, 70, or 30.9 per cent, occurred first in the anterior cell. It may be that the initial radiation of the two dividing cells affects the anterior cell more strongly, and consequently the posterior cell divides earlier more frequently than does the anterior cell.

Relative susceptibility of the various cells in chains of two's, three's, and four's.—When lethal doses of ultra-violet radiation are applied to dividing pairs, the cell which is actually undergoing division disintegrates first. This result is entirely in accord with the findings of earlier workers in the study of embryonic cell division in such forms as *Arbacia*, *Planorbis*, and *Ascaris*. (In this connection, see Lillie, 1916; Richards, 1922; and Ruppert, 1924.) Also, the anterior cell of fully formed chains of three's and four's appears to be more susceptible than the more posterior cells. Such a situation is pictured in Figure 8. Here the first three cells of a 5-day old four-celled individual disintegrated, while the fourth cell remained normal up to the time of fixation. The disintegration gradient was easily watched in this specimen, and the progress of disintegration interrupted at the desired stage.

Behavior of chains of three and four cells.—One of the four-celled specimens was maintained intact and active for a period of 5 days, and during this time its swimming habits were studied. It was noticed that after the first day a greater degree of anteroposterior control and co-ordination developed. At the outset, following recovery from the effects of radiation, there was the typical counterclockwise forward spiral. After this, a peculiar eel-like swimming was established. An undulating forward movement was apparently initiated and maintained by the anterior cell. This anterior cell periodically adhered to the slide; and by a peculiar pull first on one side and then on the other, it appeared to try to "wag off" the other three cells. Not succeeding in doing this, it swam away but continued the same motion, the connection between it and the second

cell acting as a hinge. The second and third cells were well connected by a wide cytoplasmic bridge; and the third and fourth, by a narrower one. Granules could be seen to pass from one cell to the other. The specimen was radiated at 12 hours and at 36 hours after the first radiation of the two-cell stage, to prevent further division. On the fourth day, the whole chain of four cells appeared less active than at any time previously. The anterior cell was occasionally pushed along and often bent back on itself. Co-ordination and transmission were reduced. Striking the anterior cell against an obstacle no longer caused an immediate reversal of the entire four cells, as in the typical avoiding reaction of the individual *Paramecium*, and as previously exhibited by this specimen and by other chains of two's, three's and four's. Contact of the second cell, however, with the same or another obstacle, caused a typical reversal. It was noted that the anterior cell had become more and more pinched off from the second cell and that there was no longer any visible protoplasmic connection between them. Apparently the cuticle alone was unable to function in transmission and co-ordination.

On the fifth day, the anterior cell had lost its turgor, appeared flaccid, and was powerless to control the movements of the whole. It appeared almost devoid of granules. The entire chain of cells was very inactive. It was hoped that a slight dose of ultra-violet radiation might stimulate the cells to greater activity, but the stimulation was too transitory to be of any apparent benefit to the chain of cells. A second dose of radiation proved fatal. Death by cytolysis appeared first in the anterior cell and then in the second and third cells successively. At this point the specimen was fixed and mounted. Other chains of four cells have shown the same susceptibility gradient to lethal doses of radiation, suggesting an inheritance of relative regional differences in susceptibility along an anteroposterior axis, at least to the third generation.[1] Petersen (1927) has found a similar inheritance of differences in division rates.

In susceptibility experiments with three-celled individuals, where two of the cells have just resulted from a division of one of the original conjoined daughter cells of a pair, it was found that the products

[1] Others who have presented evidence for axial gradient relations in *Paramecium caudatum* are Bills, 1924; Child and Deviney, 1926; De Garis, 1928; and Hertel, 1904.

of the recently divided cell were most susceptible to lethal doses of radiation. Obviously, a relatively high rate of division parallels (or perhaps is the basis of) a high degree of susceptibility. Petersen (1927) also correlates division rate with metabolic activity.

As previously stated, cells which are actively dividing are more susceptible to large doses of radiation. A similar high degree of susceptibility has been found in dividing *Arbacia* eggs by R. S. Lillie and others. Lillie (1916) found a rhythmic susceptibility to hypotonic sea-water, corresponding to the period just before and during the formation of the cleavage furrow in the dividing egg (see, also, Just, 1928).

When sublethal doses of radiation were used (i.e., doses just heavy enough to produce a cessation of movement and a settling to the bottom of the dish), dividing cells and newly formed pairs recovered their powers of swimming more quickly than did non-dividing cells. This is probably to be correlated also with their higher rate of metabolic activity and their greater power of recovery. Child has repeatedly demonstrated that cells of higher physiological activity recover more rapidly from exposures to sublethal doses of agents than do less active cells or regions of the organism.

Monster formation.—Calkins (1925 and 1926), De Garis (1927), and others,[1] have described the occurrence of protozoan monsters produced experimentally or found in cultures. In these experiments, two types of monsters have been produced: the axiate kind figured by Calkins (1926, p. 493) and De Garis (1927, p. 147), and the anaxiate forms which have a great many more or less complete and closely amalgamated components (see, also, Calkins, 1926, p. 203, fig. 487). Frequently cells divide irregularly—i.e., the two daughter cells are not the same size, and one or the other may not be quite normal in appearance. A normally dividing cell may come to rest at the bottom of the dish, and the two daughter cells may make an angle with each other instead of having a continuous axis. Possibly the side which at the time of exposure to ultra-violet radiation is turned toward the lamp, may be more affected, and growth on that side may proceed less rapidly. At any rate, when swimming is recovered,

[1] For a review of the literature on monster formation in Protozoa, see De Garis, 1927.

such a pair of cells, due to the distorted axis, may spin around in place or progress in exaggerated spirals (see Fig. 7).

In such forms, the nuclear apportionment between the cells may or may not be equal. In many instances the nuclear division appears to lag behind the protoplasmic division, indicating a greater susceptibility in the nuclear material to highly toxic doses of radiation. Similar differences in susceptibility have been pointed out in other forms, particularly in studies with radium and X-rays.

When such an irregular pair of cells divides again, a monster is formed. Cells divide off from the original pair at irregular angles, and may or may not leave the parent cells, depending on their degree of complete development. In this way forms are produced which may consist of a dozen or more cells, each with a more or less complete complement of cell characters. Usually when the products of division remain attached, they are distinctly abnormal. Forms of this sort have been kept alive for 6 days following the radiation of the original pair. De Garis (1927) reports the production of monsters similar to those described by Calkins (1926, p. 493, Fig. 205) and attributed by him to "old age" of the culture. (See, also, De Garis, 1927, p. 147, for the history and figures of a 12-day old specimen experimentally produced by KCN.) Such monsters as these, with a definite axis anteroposteriorly through the chain of irregular cells, are said by Calkins never to grow into the amorphous type of monster.

In the experiments with radiation also, this appears to be true, provided the axiate mass is kept under favorable conditions. It is conceivable, however, that further inhibition may eliminate the axial relations in such a form and that further division may occur in any axis. Such extremely unfavorable conditions may be conceived of in "old-age" cultures. In the experiments with ultra-violet radiation, it seems that the greater the irregularity of division of the two daughter cells following original radiation of the dividing cell, the greater is the chance for the production of large amorphous masses.

It may be pointed out in passing, that when *Paramecia* are exposed *en masse*, as in the earlier work reported in this paper, larger doses of radiation are necessary to kill than when individuals are exposed singly or in smaller groups (a more complete study of mass

protection in *Paramecium* is being made by W. Petersen in the Department of Zoölogy of the University of Chicago and will be reported by her). It is interesting to note that radiated pairs kept with other radiated single animals do not remain paired in as great numbers as when separated or kept with other tandem pairs. Apparently they are stimulated by the onset of division in the single animals to complete their own division cycle. However, "old" pairs, or monsters which will not divide further, appear to be benefited by the presence of other single cells and remain active for a longer period.

It appears from the experiments reported above that dosage is a factor in the modification of the rate of cell division. Short doses increase the rate, and longer doses decrease it. Longer exposures made before division so slow down the normal rate of physiological activity that the onset of division is greatly delayed, division slowed, and the following interdivisional period lengthened. At the end of the first day a recovery in rate of physiological activity occurs, and the rate of division gradually approaches the normal rate. Similar effects have been observed with other physical and chemical agents.

Where the degree of inhibition is great during early division stages, the process of division is delayed and may remain incomplete, as in the tandem pairs, three's, and four's. Non-dividing cells may be kept from dividing for a period of days by exposures made at intervals of twice a day, i.e., before recovery from each exposure can be complete. The animals are thus kept in a constant state of non-division which is the result of a succession of alternate inhibitions and partial recoveries.

Calkins describes a condition of rejuvenation through cell division as follows: "The protoplasm is restored to the labile condition of a young organism" at "each division of the cell." For the first few days of enforced non-division, the vitality of the individuals appears not to be impaired. They are normally active, maintain their normal size, and are able to recover from each successive dose of radiation. If division is a rejuvenating process and, under normal conditions as in the controls, is necessarily a part of the life of the individual, these experiments with radiation have imposed conditions on the individual which make division impossible and apparently unnecessary, for a few days, at least. However, a waning

vitality, such as Robertson (1923) describes, appears probably "due to accumulation of substances which can no longer be discharged from the cell."

The production of "permanent" pairs by the exposure of dividing cells to radiation during early division stages is an example of inhibition with partial recovery in one generation, followed by complete recovery in the next. Although the cytoplasm appears not to be able to complete its division process, the nuclear material reorganizes to the extent of getting the conjoined cells ready for another division cycle.

So far, it has not been possible, by means of ultra-violet radiation, to produce chains of more than four cells. There are some interesting characteristics of such chains which should be mentioned here. For example, the length of the chain does not determine its behavior as an individual. Two's, three's, or four's show the same type of response to obstacles as do single cells. Dominance of the anterior cell over the other cells appears to grow with increasing age during the period of maximum activity of the chain. Furthermore, the degree of protoplasmic continuity is a factor in maintaining such dominance. When the vitality of the chain begins to wane, the anterior cell is the first to show signs of decadence, and may be dragged along by the other cells or even be resorbed, as was the case in one specimen in which, on the fourth day, the anterior cell had become nothing more than a knob.

SUMMARY

1. Ultra-violet radiation may stimulate or inhibit the rate of division in *Paramecium caudatum*. Dosage and physiological condition of the animal are factors.

2. Inhibiting doses may be applied at any stage of the division cycle or during the interdivisional period. The greatest effect for a given dosage is obtained by exposures made during the early stages of division.

3. The division process may be retarded to the extent of failure of completion. This leads to the formation of tandem pairs of normal cells which fail to separate. Each of these cells may again divide, and division be again arrested by radiation, resulting in the formation of "permanent" three's and four's.

4. Successive exposures may entirely prevent division for a number of days.

5. Chains of two's, three's, and four's exhibit the characteristic behavior of single cells. Dominance and subordination appear, and an anteroposterior gradation of susceptibility to lethal doses of radiation may be demonstrated.

6. It is conceivable that the evolution of multicellular forms may have been brought about by an inhibition of cell division followed by a partial recovery and further incomplete divisions in which the division products fail to separate. The appearance of an anterior dominance would impose the necessary conditions for individuality in such an organism.

LITERATURE CITED

BILLS, C. E. 1924. Some effects of the lower alcohols on *Paramecium*. Biol. Bull., XLVII, 253.

BOVIE, W. T., and HUGHES, D. M. 1918. The effects of quartz ultra-violet light on the rate of division of *Paramecium caudatum*. Jour. Med. Res., XXXIX, 223.

CALKINS, G. N. 1925. *Uroleptus mobilis*. V. The history of a double organism. Jour. Exp. Zoöl., XLI, 191.

———. 1926. The biology of the Protozoa. New York: Lea and Febiger.

CATTLEY, R. 1909. Cytological changes produced by the action of X-rays on growing root tips of plants. Jour. Path. and Bact., XIII, 380.

CHILD, C. M., and DEVINEY, E. 1926. Contributions to the physiology of *Paramecium caudatum*. Jour. Exp. Zoöl., XLIII, 257.

DE GARIS, C. F. 1927. A genetic study of *Paramecium caudatum* in pure lines through an interval of experimentally induced monster formation. *Ibid.*, XLIX, 133.

———. 1928. The effects of anterior and posterior selections on fission rate in pure lines of *Paramecium caudatum*. *Ibid.*, L, 1.

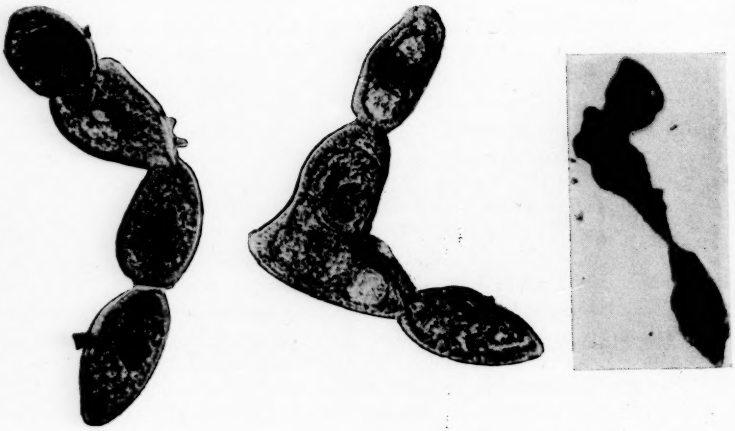
HERTEL, E. 1904. Über Beeinflussing des Organismus durch Licht, speziell durch die chemische wirksame Strahlen. Zet. f. Allg. Physiol., IV, 1.

HINRICHS, M. A. 1924. A study of the physiological effects of caffein upon *Planaria dorotocephala*. Jour. Exp. Zoöl., XL, 271.

———. 1927. Ultraviolet radiation and the fertilization reaction in *Arbacia punctulata*. Biol. Bull., LIII, 416.

JUST, E. E. 1928. Studies of cell division. II. The period of maximum susceptibility to dilute sea-water in the cleavage cycle of the fertilized egg of *Arbacia*. Physiol. Zoöl., I, 26.

1 2 3 4 5



6 7 8

LILLIE, R. S. 1916. The physiology of cell division. VI. The rhythmical changes in the resistance of the dividing sea-urchin egg to hypotonic sea-water and their physiological significance. Jour. Exp. Zoöl., XXI, 369.

MARKOVITS, E. 1922. Zytolitische Veränderungen von Paramecium nach Bestrahlung mit Mesothorium. Arch. f. Zellf., XVI, 238.

PACKARD, C. 1916. The effect of radium radiations on the rate of cell division. Jour. Exp. Zoöl., XXI, 199.

———. 1924. The susceptibility of cells to radium radiations. Biol. Bull., XLVI, 165.

PETERSEN, W. 1927. The axial gradient in *Paramecium*. Science, LXVI, 157.

RICHARDS, A. 1914. The effect of X-rays on the rate of cell-division in the early cleavage of *Planorbis*. Biol. Bull., XXVII, 67.

———. 1914. The effect of X-rays on the action of certain enzymes. Amer. Jour. Physiol., XXXV, 224.

———. 1922. The acceleration of the rate of cell division. Biol. Bull., LXIII, 348.

ROBERTSON, T. B. 1923. The chemical basis of growth and senescence. Philadelphia: J. B. Lippincott Co.

RUPPERT, W. 1924. Empfindlichkeitsänderung in des Ascariseies auf verschiedenen Studien der Entwickelung gegenüber der Einwirkung ultravioletten Strahlen. Zeit. f. wiss. Zoöl., CXXIII, 103.

EXPLANATION OF FIGURES

Figures 1–8 represent photomicrographs of mounted two-, three-, and four-celled *Paramecium caudatum*. Complete division was prevented by exposure to ultra-violet radiation during early stages in the division cycle.

FIGS. 1 and 2.—Two-celled animals. Figure 1 shows an early stage in the division cycle, shortly after exposure to ultra-violet radiation. Figure 2 shows a later stage (24 hours after radiation), in which both cells are still connected.

FIGS. 3–5.—Three-celled animals. In each case a second division has occurred in the posterior cell of the conjoined pair. Early stages of the second division are shown.

FIGS. 6–8.—Four-celled animals. Figure 6 shows an individual in which the posterior cell divided slightly earlier than the anterior cell. In the individuals represented in Figures 7 and 8, division occurred practically simultaneously in both cells. Figure 7 shows irregular division in the two middle cells, which are products of a first division. The second divisions were both regular. Such a specimen swims around in a spiral but makes little or no forward progress. Figure 8 represents a four-celled individual which had been exposed to a lethal dose of ultra-violet radiation. The anterior three cells are shown disintegrating before the posterior cell is attacked.

ON THE PHYSIOLOGY OF THE PALLET MECHANISM OF THE SHIPWORM, TEREDO NAVALIS

HAROLD F. BLUM

Rudolph Spreckles Physiological Laboratory, University of California, and the Department of Animal Biology, University of Oregon

THE pallet mechanism of the Teridinidae, or shipworms, offers a very interesting example of adaptation to environment. It is, perhaps, particularly important in *Teredo navalis*, which inhabits brackish water where sudden changes in the salinity of the water may frequently occur. The animal meets the advent of water of unfavorably low salinity by closing its burrow with the pair of calcareous pallets attached to the mantle near its posterior end. At the same time the excurrent and incurrent siphons are retracted within the burrow. The importance of this mechanism in the survival of the organism has been shown by the writer (1922).

Experiments were made on specimens of *T. navalis* which had been grown in wooden blocks suspended in the waters of Carquinez Straits, at Crockett, California. The blocks were removed from the straits water for only a short time before the experiments were performed. Immediately after such blocks are removed from the water, the siphons are found withdrawn into the burrows. However, if the surface of the block remains wet with a film of water the siphons soon extend themselves into this film, remaining there as long as the surface remains wet and they are not disturbed. I often observed this extension of siphons on the surface of pilings taken from the dock of the California and Hawaiian Sugar Company at Crockett, during the spring of 1922. Great variations of the salinity of the straits water occurred at this time, and it was noted that the siphons were only extended when the salinity was above the lethal point (5 o/oo).

In these experiments, the blocks, after removal from the water, were dried with filter paper. This caused the retraction of all the siphons. The pallets of a single organism could then be caused to withdraw, and the siphons to extend, by placing a drop of sea water over the opening of an individual burrow. The movements of the

siphons and pallets were readily observable by means of a binocular dissecting microscope. The drop of water was prevented from spreading by a ring of vaseline constructed around the mouth of the burrow. If the excess of sea water in the drop was carefully taken up with a piece of filter paper, the siphons remained extended on the surface of the wood. It was necessary in removing the water to avoid touching the siphons with the filter paper, as mechanical stimulation causes them to retract rapidly. If the sea water was now replaced by a drop of water of salinity below 5 o/oo, the siphons were retracted and the pallets approximated to close the mouth of the burrow. The volume of the drop of water was not appreciably altered before or during this response. This fact indicates that the stimulus bringing about these movements occurs locally, since the water is not drawn into the mantle cavity in sufficient amount to affect any distant organs. It has been rather generally assumed on morphological grounds that the osphradia act as chemoreceptors to test the water which passes over the gills (Lang, 1896, p. 163). Sigerfoos (1907) assumes the possibility that the stimulus from unfavorable salinity which causes the retraction of the siphons occurs at this point. If this were true, it would necessitate drawing a considerable volume of water into the mantle cavity. This does not occur, however, as is shown by the foregoing observation. Also, the writer (1922) has shown that the *Teredo* is very susceptible to water of low salinity if exposed to it directly and that the protection afforded by the pallet mechanism lies in the prevention of the access of such water by the closing of the burrow mouth. The efficient functioning of this protective mechanism would seem to necessitate the reception of the stimulus at some point near the entrance of the burrow.

The co-ordinated action of the two structures—namely, the pallets and the siphons—suggests a reflex mechanism involving a single set of receptors. The siphons, readily responsive to mechanical stimuli, would appear to be the most probable site of such receptors. Certain facts, however, indicate that these are at least not the only organs concerned in the reception of the stimulus. When teredos are exposed to water of low salinity (below 5 o/oo) for a long period, the siphons remain completely retracted and the burrows closed by

the pallets as long as the salinity remains low. If after some days these teredos are exposed to water of favorably high salinity, the siphons are extended within a very few minutes (Blum, 1922). This would indicate that the receptors are located elsewhere than in the siphons, possibly in the collar which surrounds the pallets.

In my experiments I have observed another interesting phenomenon which supports this view. Sometimes when siphons which had been extended in a drop of sea water were exposed to a drop of distilled water, the siphons were apparently not retracted but remained outside the burrow in the drop of water. In such cases closer examination always showed that the siphons were floating free in the drop, having been cut off by the closure of the pallets. This "autotomy" is apparently due to some inco-ordination of the siphon-pallet mechanism, the pallets approximating before the siphons are completely retracted. After this "autotomy" had taken place, the pallets could still be caused to retract, and the stumps of the siphons to extend, by placing a drop of sea water upon the opening of the burrow. In such instances, no part of the siphons, or only damaged remnants, were exposed to the sea water, yet the stimulus was received and the appropriate response brought about.

SUMMARY

1. The protective pallet-siphon response in *T. navalis* is brought about by stimulus of receptors located somewhere in the region of the pallets and siphons, and not in the osphradia.

2. These receptors are probably not located exclusively in the siphons.

3. "Autotomy" of the siphons may be caused by sudden exposure to water of extremely low salt content. This occurs when the pallets close before the siphons are completely retracted.

LITERATURE CITED

BLUM, H. F. 1922. On the effect of low salinity on *Teredo navalis*. Univ. of Calif. Publ. in Zoöl., XXII, No. 4, 349–68.

LANG, A. 1896. Text book of comparative anatomy (English translation). London.

SIGERFOOS, C. P. 1907. Natural history, organization, and late development of the teridinidae, or shipworms. Bull. Bur. Fish., XXVII, 191–231.

AXIAL DEVELOPMENT IN AGGREGATES OF DISSOCIATED CELLS FROM CORYMORPHA PALMA

(Fifty-two figures)

C. M. CHILD
Hull Zoölogical Laboratory, University of Chicago

DURING the summer of 1927 the method of cell dissociation was applied to *Corymorpha* in order to determine, first, whether aggregation of the cells would occur; secondly, if aggregation did occur, whether the aggregates would develop into individuals or partial forms; and thirdly, whether anything could be learned from the aggregates concerning the origin of polarity and symmetry. Certain of the results of this investigation are presented in this paper. In this connection I desire again to express my appreciation to the director of the Scripps Institution of Oceanography, Dr. T. Wayland Vaughan, for making this work possible by placing the facilities of the station at my disposal.

METHOD

Except for a few experiments with hydranths, the naked regions of the columns or stems were used in all cases; and since comparison of animals of different sizes or ages was not attempted, these were taken from the largest animals occurring in the summer, which were 40–60 mm. in length. After isolation from the rest of the body, the stem pieces were ground in a mortar with thoroughly washed sea sand. Care must be taken to remove all excess water, otherwise some of the stems escape dissociation. Five to ten seconds of grinding is usually sufficient: if grinding is continued too long, little or no aggregation occurs, all the cells being presumably injured or destroyed. After grinding, 50–100 cc. of filtered sea water are added, the sand is stirred gently to permit the particles to become suspended in the water, and the fluid is then filtered through bolting-cloth, 150 meshes to the inch.

This method of grinding with sand was used for dissociation in preference to that of pressing the cells through bolting-cloth, which

has been used for various sponges (H. V. Wilson, 1907, 1910*a*, 1910*b*, 1911; Müller, 1911; Huxley, 1921; Galtsoff, 1925) and some hydroids (H. V. Wilson, 1911; Föyn, 1927), because the cells of *Corymorpha* are rather firmly attached to the somewhat tough mesogloea and are not as readily dissociated by mere pressure as are the cells of sponges and of smaller, more delicate hydroids. Moreover, a more complete dissociation was attained by grinding than by pressure. The meshes of the bolting-cloth were about 0.1 mm. in diameter; but the particles, after grinding, were mostly much smaller than this, their size depending largely on the amount of grinding to which the stems were subjected. In most experiments, very few of the fragments were more than 30–40 μ in diameter, and most of them were smaller. The fluid, after passing through the bolting-cloth, appeared merely somewhat milky to the naked eye.

The dissociated cells or cell groups approach spherical form rapidly, so that within a few minutes after dissociation they are in the form of small balls, and these aggregate into larger masses. During the first 4 or 5 hours after dissociation at a temperature of about 22° C., the particles adhere to each other when they come into contact, and so undergo a progressive aggregation, and the aggregates gradually become spherical. Since this capacity for aggregation decreases from the time of dissociation and completely or almost completely disappears within 4–5 hours, it is desirable for purposes of aggregation to bring the particles into contact with each other as rapidly as possible. Since no centrifuge was available and since the dissociated particles are heavy enough to settle in sea water, the procedure found most satisfactory, after various experiments, was to bring the bolting-cloth filtrate into small slender test tubes for settling. In these tubes the particles aggregated as they came into contact during settling and after they had reached the bottom; and in most cases after 1–2 hours they had formed irregular masses or flattened sheets 2–3 mm. in diameter or, if the cellular material was abundant enough, a single sheet covering the bottom of the tube, that is, 6–8 mm. in diameter.

Left undisturbed, the aggregates gradually approached spherical form; but since it was found that masses larger than 1 mm.

after the approximately spherical form was attained showed a high death rate or lost a considerable part of their cells before developing, the larger masses and sheets were cut into pieces as desired in the later experiments, some sheets being cut into sixty pieces, almost all of which developed. The aggregates or pieces of desired size from larger aggregates were placed in stender dishes in filtered sea water, which in most experiments was changed daily or oftener.

In the experiments on the influence on polarity and development of the differential of position certain lots remained as completely as possible without change of position, while others were repeatedly moved about and turned over. Change of water without disturbance was accomplished, when necessary, by a small siphon with flow regulated by a screw clamp. With a very slow rate of flow most of the water could be removed and fresh water added without changing the positions of the aggregates. Change of position was accomplished in some cases by currents from a pipette; in others the individual aggregates were turned over with a needle.

The aggregates were brought into the stender dishes within, at most, 5–6 hours after dissociation, and before they had become spherical. Some were irregular flattened masses in the form in which aggregation had occurred; in other cases large sheets were cut into pieces of more or less uniform size. In all cases the time when the experiment was begun was afternoon. The lots to be moved were moved once or twice on that first day, then were allowed to remain over night without disturbance, and after that were moved in most cases twice a day, morning and evening—in some cases only once a day. All lots remained undisturbed from 9:00–10:00 P.M. to 7:00–8:00 A.M. The results showed that while such frequencies of change of position are highly effective in determining the forms which develop, the undisturbed periods are apparently sufficient to permit the effects of differential exposure to appear, even in the moved lots. Further experiment with much more frequent or continuous change of position in moving water is planned for the future.

In general, the death-rate was much higher in the undisturbed than in the moved lots, even with daily change of water. Death and disintegration began in the regions in contact with the substratum; and intact portions could often be saved by removal from

the disintegrating parts and change of position but, of course, in such cases could no longer be listed as undisturbed.

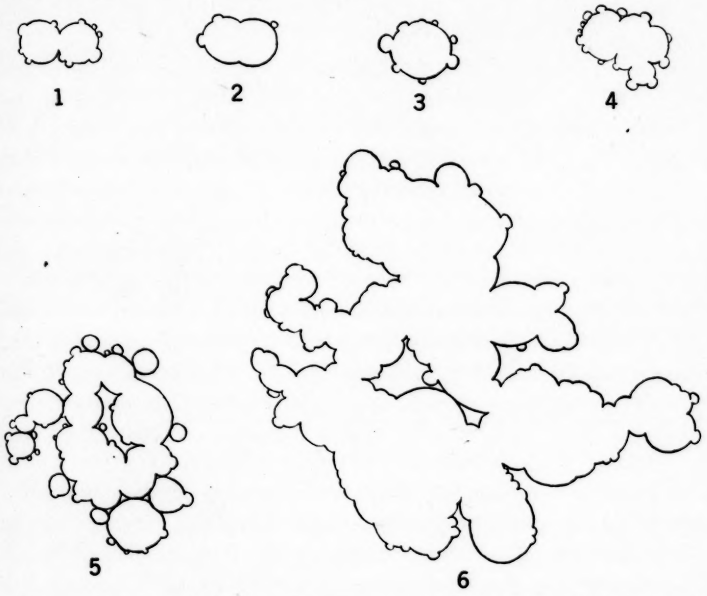
AGGREGATION OF CELLS FROM THE NAKED REGIONS OF STEMS

In all cases, so far as observed, the large entodermal parenchymal cells were destroyed by the grinding, the persisting cells or cell masses being from the ectoderm and from the entoderm of the longitudinal peripheral canals. These entoderm cells differ but little in size from the cells of the ectoderm, and it has not been found possible thus far to distinguish the cells of the two layers with certainty after dissociation. Aggregation proceeds very rapidly at first, more slowly later; and each aggregate gradually approaches spherical form.

Under microscopic observation on a glass substratum the small cell masses often show slight and slow movement with slight change of shape, but distinct amoeboid processes have not been seen. No evidence has been found thus far to indicate that masses within a certain distance exert any chemotactic or other effect on each other which brings about their approach and fusion. Many cases of approach and fusion have been observed; but in many other cases, in which the original distance was no greater, no approach took place, and in still others, one or both of the masses moved so that the distance between them increased. Often one mass approached another until almost or apparently actually in contact; then reversal of direction of movement took place and led to separation. In short, the observations thus far indicate that contact and fusion are largely if not wholly the result of chance.

Contact soon after dissociation is usually followed by fusion, and in the course of 5–10 minutes two small approximately spherical masses may undergo complete fusion (Figs. 1–3). As the larger masses appear in consequence of continued fusion, numerous smaller masses and particles are seen on their surfaces. These are smaller cell-masses or perhaps single cells which are gradually being incorporated into the larger masses. Figures 4 and 5 show further stages in aggregation, such as may appear within the first half-hour after dissociation; and Figure 6 shows a flattened irregular sheet, approximately 3×3 mm. in diameter, as it appeared 5 hours after dissocia-

tion. Twenty-four hours later this sheet had become spherical, and still later it developed into a complete individual. When first formed, all the masses, large or small, are solid; and the smaller—0.3 mm. or less—are sufficiently translucent so that the uniformity of structure throughout is evident, as might be expected. At this time ectoderm and entoderm are, of course, not distinguishable as



FIGS. 1–6.—Stages of aggregation. Figs. 1–3, successive stages in fusion of two small aggregates with still smaller masses aggregating on their surfaces. Figs. 4 and 5, later stages of aggregation. Fig. 6, a flattened irregular sheet about 3×3 mm. in diameter 5 hours after aggregation.

definite layers; nor has it been possible to distinguish ectoderm and entoderm cells in the aggregates. The readiness with which aggregation occurs and its rapidity decrease from the time of dissociation; and after 4–5 hours in sea water very little or no further aggregation occurs, even when the masses remain in close contact with each other for hours. Evidently the exposure to sea water has brought about a gradual change of the surfaces of the masses so that their proto-

plasms no longer adhere and become continuous on contact. Probably this change consists essentially in the formation of a precipitation membrane. The presence of such a membrane is indicated by the more definite and more highly refractive appearance of the surface of the masses in optical section after a few hours, as compared with earlier stages.

RESULTS OF DISSOCIATION OF HYDRANTHS

In a few experiments the hydranths removed from the stems used for dissociation were also ground with sand, after complete removal of stem regions. It became evident at once that the cells of the hydranth behave very differently from those of the stem, in that dissociation is followed by the aggregation of only a few cells into solid masses resembling those formed by stem cells. Since hydranth dissociation was found to be unsuited to the chief purpose of the investigation (viz., the study of development and particularly of polarity in the aggregates), comparatively little attention was devoted to it. The following observations made on the hydranth material indicate the chief differences between it and the stem cells. As in the case of the stem dissociations, the bolting-cloth filtrate was brought at once into small test tubes for settling. After 3–4 hours the sediment at the bottom of the tube contained a few small solid masses like those found in dissociated stem material, but none were larger than 0.1 mm. in diameter. Most of the dissociated material was in the form of loose accumulations of cells and cell débris, apparently held together mainly by slime, and readily separated by slight currents. Twenty-four hours after dissociation a few of the solid spherical masses similar to those formed by stem cells were found; but there had been no further aggregation, and the loose accumulations of cells showed no indications of aggregation. In later stages these accumulations not only failed to aggregate, but when left undisturbed, the cells or cell groups gradually migrated out from the central accumulations. Three days after dissociation protoplasmic strands and networks were found spreading over the substratum, often anastomosing. Continued observations showed slow amoeboid change of shape in many of the cell groups. The groups contained nematocysts, often in considerable numbers; but the liv-

ing, intact cells had apparently freed themselves from the cell débris which had been left behind. In addition to the cell groups, many single free cells were observed, some, perhaps all, of which were amoeboid. Some merely underwent slow changes of shape, while others showed definitely directed locomotion. These latter possessed a characteristic form, the advancing front being broad and rounded and behaving as a single pseudopod, while the posterior end was more or less pointed (Fig. 7). The granules were confined to the posterior third or half of the length of the cell, the anterior portion being hyaline. These cells may perhaps be parasites rather than hydranth cells. At this time the small solid aggregates were no longer found.

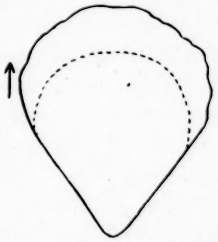


FIG. 7.—An amoeboid cell from dissociated hydranths. Arrow indicates direction of locomotion. The broad rounded portion anterior to the broken line is hyaline; the remainder, granular.

Five days after dissociation, still further migration had occurred; but no movement could be observed, and some of the cells were apparently dead and stained readily with eosin. Six days after dissociation, all were apparently dead. These observations show, at least, that most of the cells of the hydranth do not aggregate into solid masses as do the cells of the stem, though they may remain more or less in continuity and show amoeboid activity either as single cells or as larger groups. Probably the comparatively few small aggregates which do form from hydranth cells represent the less highly specialized regions of the hydranths. It may be that their cells come from the early developmental stages of the gonophores which continue to develop as buds, even in large old hydranths.

THE DEVELOPMENT OF THE AGGREGATES

The approach to spherical form continues in all aggregates, large and small, and even in those formed as thin sheets. Within 24 hours or less after dissociation all except some very large sheets 6–7 mm. in diameter have become spherical or approximately spherical solid balls, their size depending on the original size of the aggregate or the size of pieces into which larger aggregations were cut.

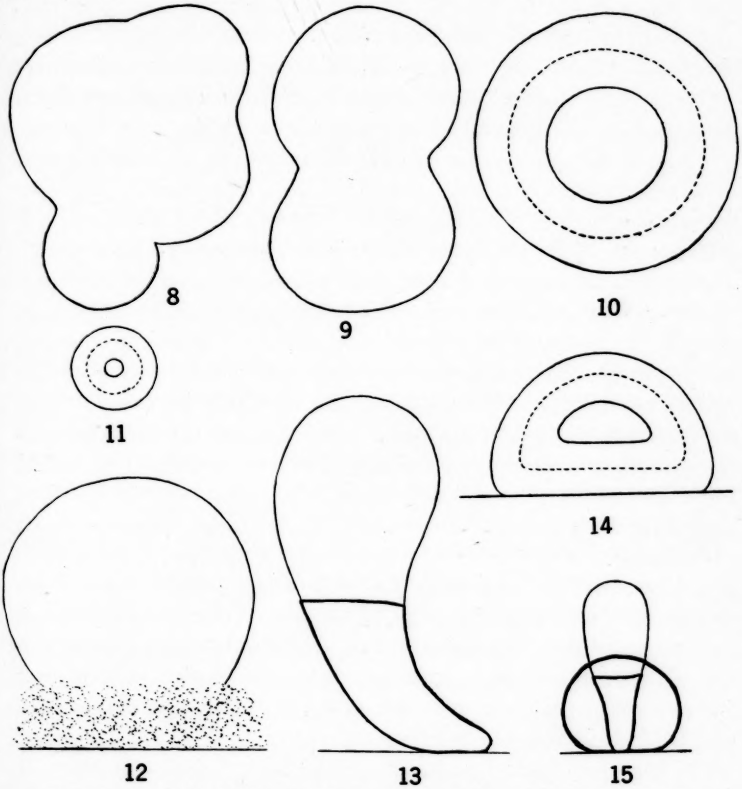
The largest sheets, conforming in curvature to the bottoms of the tubes in which they were formed, became cup-shaped or approached hollow spheres by increase of the curvature already present; but thus far all such large aggregations have died without further development.

The smaller masses, 0.5 mm. or less, approximate very closely the spherical form; but some of the larger masses remain somewhat irregular in form, appearing as if the final fusions were not quite completed (Figs. 8 and 9). These irregularities of form are gradually eliminated in the course of development.

Within 36–48 hours after dissociation, some of the masses begin to show distinct ectoderm, mesogloea, and entoderm in normal relations and volumes, and at about this stage a definite enteric cavity usually appears (Figs. 10, 11). At this time, also, slight contractile changes of form begin to occur in the more advanced aggregates. Nothing positive can be said concerning the origin of ectoderm and entoderm, for histological study of the aggregates has not yet been made, and the observations on the living material, while suggestive, are not finally conclusive. The various possibilities are considered below in the general section of this paper (pp. 449–50).

The larger aggregates, 0.5 mm. or more in diameter, are distinctly less viable than the smaller as long as the spherical form persists. Their death-rate is higher when they are left undisturbed on the bottom of the container than when they are periodically moved about and turned over. The higher death-rate of the larger aggregates is apparently chiefly a matter of the relation of surface and volume. The larger the solid aggregate, the greater the volume of protoplasm in relation to the surface. Apparently, in such masses adequate respiration is often impossible. When these large masses are left undisturbed, death begins in the regions in contact and adjoining the substratum, the first indication being that these regions begin to lose cells or to separate into cells and become more or less surrounded by free cells and cell débris (Fig. 12). If the position is not changed, the disintegration of the lower levels brings the adjoining regions under the same unfavorable conditions, and death may gradually progress over the whole aggregate. In many cases, however, development of the upper levels may occur even though

more or less necrosis is taking place in the regions in contact, and an individual may finally develop from the living portions of the aggregate.



FIGS. 8–15.—Early developmental stages of aggregates. Figs. 8 and 9, incomplete fusions. Figs. 10 and 11, two-layered, spherical forms; ectoderm and entoderm are present as definite layers separated by mesogloea, which is indicated by the broken line. Fig. 12, a large aggregate undergoing disintegration in the region adjoining the substratum. Fig. 13, an elongating aggregate resembling a planula. Fig. 14, early stage of an apical partial form. Fig. 15, an aggregate elongating and emerging from a perisarcal covering.

This high susceptibility of the larger aggregates disappears as elongation and development proceed; and the large individuals re-

sulting seem to be no more susceptible than the smaller, and without food live longer than the latter. Moreover, the smaller aggregates show no such susceptible period, and under reasonably good conditions rarely die. These facts indicate clearly that the frequent death of the larger aggregates is due to the difficulties concerned in adequate respiratory exchange in the large solid spherical masses, the conditions being more extreme in the regions continuously in contact with the substratum than elsewhere.

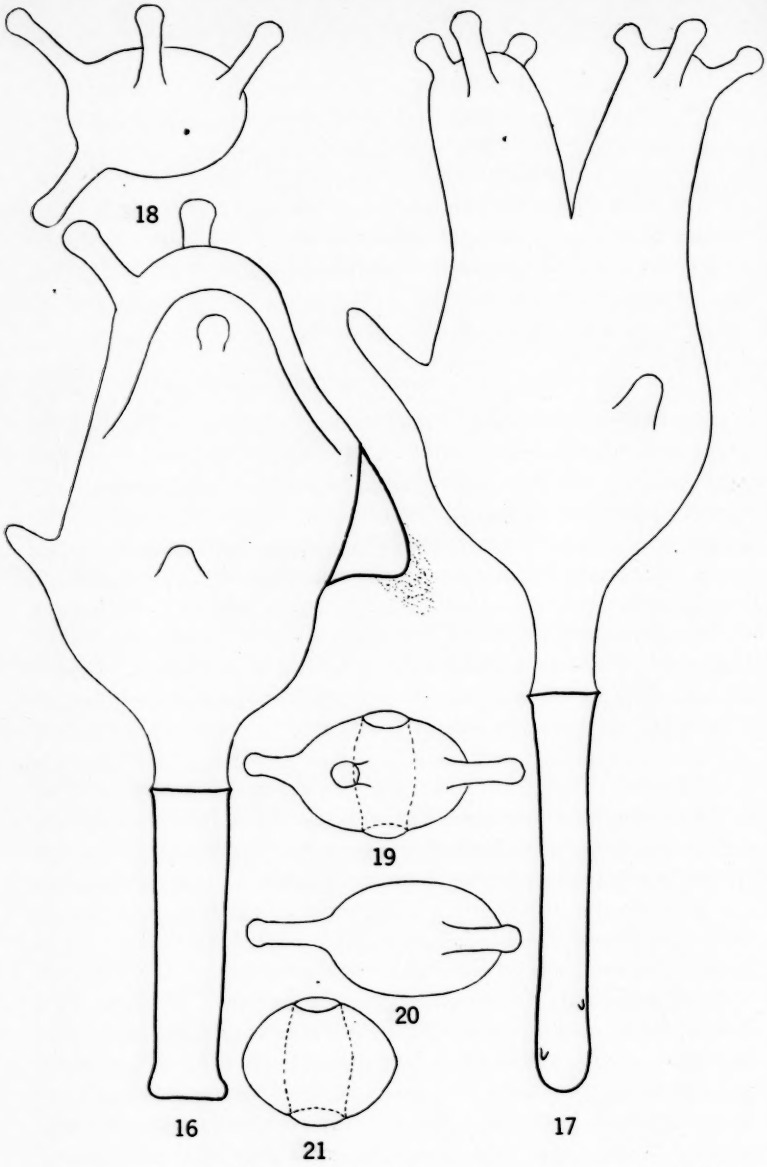
The further course of development consists in the morphogenesis of the individual or partial individual from the blastula-like, two-layered aggregate. If the aggregate develops into a complete individual with hydranth, stem, and basal region, considerable elongation occurs and the whole process is essentially identical, so far as can be determined, with embryonic development, the elongating aggregate often resembling very closely a planula (Fig. 13). In many cases, however, the proportions of the resulting individual or partial individual differ widely from those characteristic of embryonic development. The hydranth may be relatively large or relatively small in proportion to the stem and basal region, as compared with ontogeny. Great differences in size of the whole individual also occur, depending in large part on the size of the aggregate; but the size of the aggregate does not determine whether a complete or a partial individual shall develop from it. So far as can be determined, the differentiation of the various parts takes place in the same way as in ontogeny. Many aggregates, particularly the smaller, do not give rise to whole individuals, but merely to apical regions. In such cases there is commonly little or no elongation, and some flattening of the region in contact may occur (Fig. 14).

In the more rapidly developing aggregates, hydranth and stem may be clearly distinguishable, and tentacles may be appearing in 3–4 days after dissociation. In general, the smaller aggregates develop somewhat more slowly than the larger up to about 1 mm. in diameter, and aggregates much above this size have thus far died. The small aggregates (e.g., 0.1–0.2 mm. in diameter), often give rise to tentacles or to one tentacle only after seven days or more, and in many cases never develop tentacles, though they show more or less differentiation as apical regions. Aggregates less than 0.1 mm.

in diameter usually, if not always, remain spherical indefinitely and show no definite development. Forms which remain spherical may secrete perisarc over the whole surface and remain apparently apolar indefinitely. This occurs more frequently in the very small; less frequently in the larger aggregates; and even the very small aggregates often remain naked. Occasionally one of the larger aggregates which has secreted a perisarcal membrane begins later to develop, and emerges from the perisarc in the same way that the planula emerges from the egg membrane (Fig. 15).

THE FORMS WHICH DEVELOP FROM AGGREGATES

Up to the present the development of some three hundred aggregates has been observed. All of these have developed in contact with a glass substratum; but some lots have been moved about from time to time, while others have been left undisturbed. The forms which develop are classified as in earlier papers (Child, 1926*b*, 1927*a*, 1927*b*) into unipolar, bipolar, and multipolar forms, according as they possess one, two, or more than two polar axes or parts of axes. Under the conditions of the experiments thus far, very few bipolar and multipolar forms have appeared. Only five multipolar forms have been seen among about three hundred aggregates which have developed. One of these was bi-apical, with a second base developing from one side of the hydranth (Fig. 16). Its development was not followed closely, but apparently it elongated in the direction of the primary polar axis and then tipped over on one side, developing the secondary base from the region next the substratum, so that the hydranth became a dorsoventral structure. The secondary base lies between the proximal portions of the two apical ends, and its determination as a basal region may be responsible for the development of two apical ends in a plane at right angles to the dorsoventral plane instead of the normal single apical end. The four other multipolar forms were all merely bi-apical forms, essentially similar to Figure 17. All five of these forms, while actually multipolar, are predominantly unipolar. It is a fact of some interest that no cases of multipolarity involving whole hydranths or hydranths and stems, such as develop from stem pieces, particularly under the influence of inhibiting agents (Child, 1926*b* and 1927*a*), have been observed.

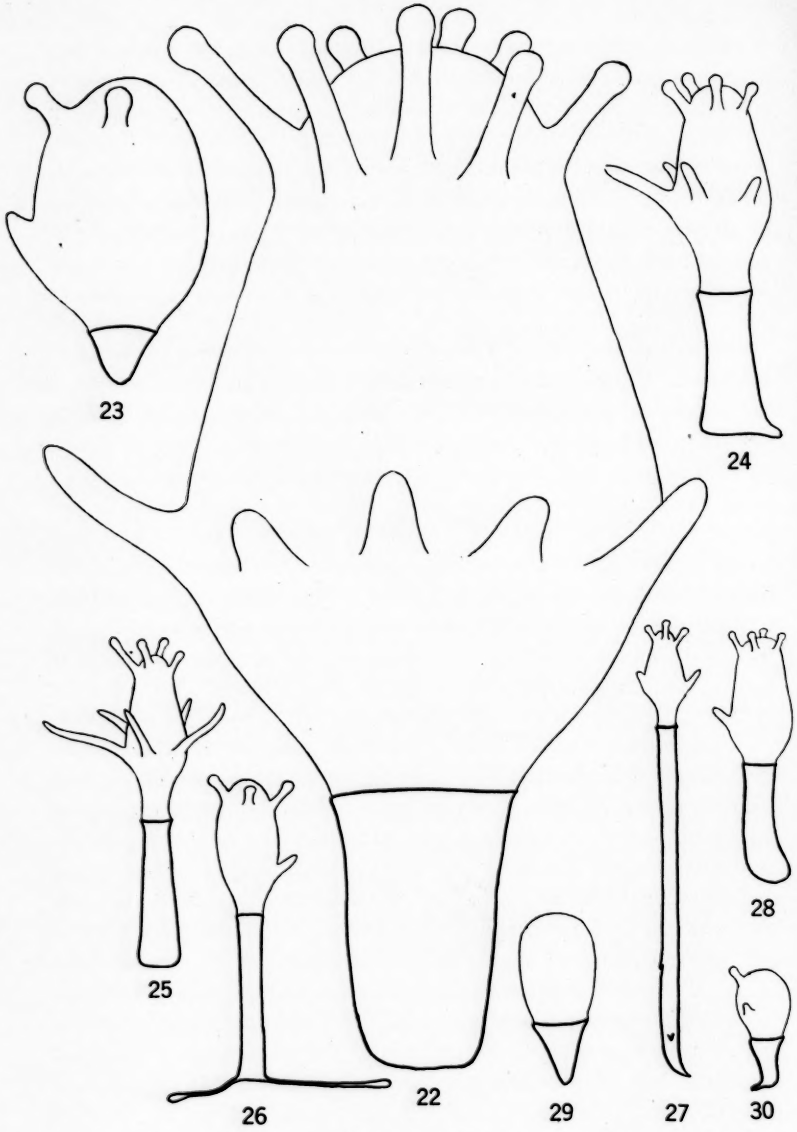


FIGS. 16–21.—Multipolar and bipolar forms from aggregates. Fig. 16, a bipolar-unipolar form in general pattern, but with a second base on side of hydranth. Fig. 17, bipolar-unipolar form; all multipolar forms except Fig. 16 were of this sort. Figs. 18–21, bipolar, apical partial forms.

Bipolar forms were also rather infrequent, so far as could be determined, and were limited to bipolar apical forms, consisting of hypostome with or without the distal portion of the manubrium. No cases of bipolar complete hydranths or hydranths with common stem region have been found. Figures 18–21 show characteristic bipolar forms. It was very difficult in many cases to determine whether the short apical forms were unipolar or bipolar. Many of the smaller of these forms never develop more than one or two tentacles; and if the mouth does not open during observation, it is not always easy to determine its position. In fact, it was found to be impossible in some cases to be certain whether or not a mouth opening had developed in some of the smaller apical forms. The entoderm could be clearly distinguished in such cases, but whether the enteron opened to the exterior could not be determined. Figure 18 is apparently bipolar with three tentacles at one pole and one at the other pole. Figure 19 has only one set of tentacles, but two mouths are present. Figure 20 shows two tentacles midway between two flattened regions, probably mouth regions, even if mouths have not broken through. Figure 21 is a bipolar hypostome region without tentacles. Figures 19 and 21 show cases in which the mouths opened widely during observation. The majority, even of the short apical forms, have apparently been unipolar. Possible factors concerned in this high frequency of unipolar forms will be considered below.

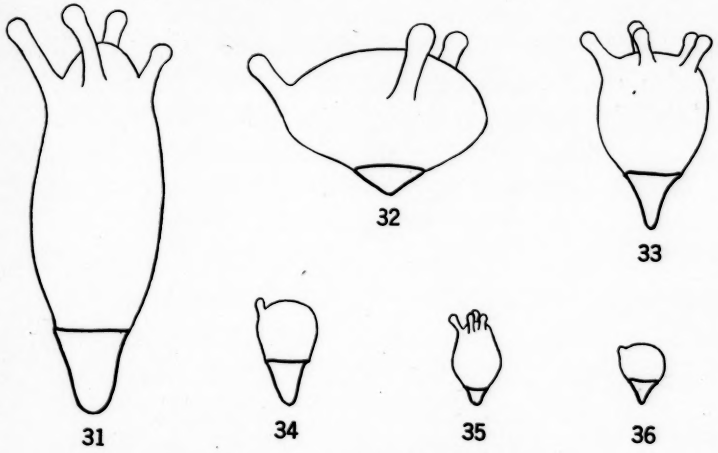
The unipolar forms fall into three rather sharply defined groups: first, complete individuals like those in nature (Figs. 22–30); second, "mosaic forms" (Child, 1926*a*, p. 776), consisting of more or less of the apical end and a base, a larger or smaller part of the intermediate regions being absent (Figs. 31–36); third, apical partial forms consisting of the hypostome alone, without distal tentacles, or with the distal region of the manubrium and distal tentacles (Figs. 37–44), or of a sector of the apical region (Figs. 45–50). As already noted, it is often impossible to determine with certainty whether the apical forms are unipolar or bipolar. This is particularly true of the sectors (Figs. 45–50), for in most of these the polar differentiation is not sufficient to show whether one pole is oral, the other aboral, or both oral.

In addition to these various forms which develop polarity, a few



FIGS. 22–30.—Unipolar complete forms of different sizes and proportions

aggregates remain apparently apolar, developing only surface-interior pattern, that is, ectoderm and entoderm in the usual relations (Figs. 51, 52). These may be completely inclosed in perisarc (Fig. 51); or some of the very small aggregates may remain naked, at least for a considerable time (Fig. 52). Apolar forms have been seen only occasionally among aggregates 0.3–0.4 mm. in diameter and never among the largest.



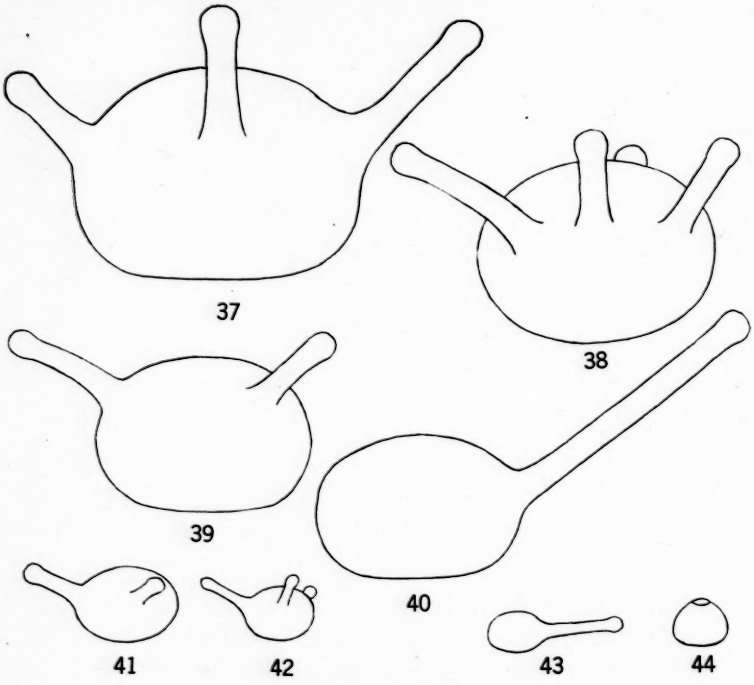
FIGS. 31–36.—Unipolar mosaic forms

All the different forms show a wide range as regards scale of organization and proportions of parts. Figures 16–52 are all drawn to the same scale of magnification (×50) in order to show these differences clearly. Before discussing the origin of such differences in scale and proportion, it is necessary to consider the influence of environmental factors on the origin of polarity and the frequency of the various forms.

THE PROBLEM OF THE ORIGIN OF POLARITY IN THE AGGREGATES

There seems to be no room for doubt that the original polarity is obliterated in the aggregates. So far as can be determined, the cells or cell masses composing any aggregate have come together purely fortuitously. No indications of selective aggregation appear,

and when the whole contents of a tube aggregate into one continuous sheet, which may be cut into pieces of any desired size, there appears to be no possibility of selective aggregation. The question whether polarity is inherent in the cells and develops in the aggregate independently of environment or whether it is determined by external

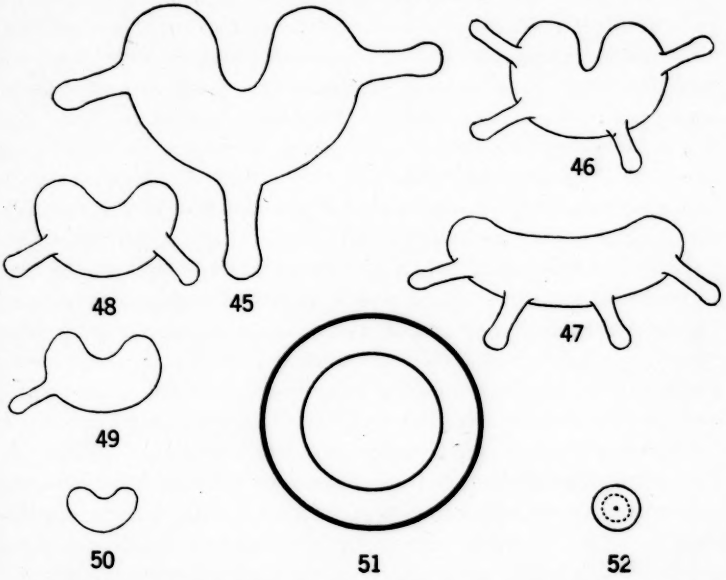


FIGS. 37–44.—Radially complete apical partial forms

conditions is one of fundamental importance. It is the question whether localization and morphogenesis can take place autonomously, that is, independently of environment and independently of preexisting regional differences in such an aggregate.

If polarity is primarily a gradient, it may conceivably be determined in some cases by differences in physiological condition in different cell groups, which have persisted from the original indi-

vidual of which they formed a part. For example, if a group of cells from a level near the distal end of the stem (i.e., the high end of the stem gradient) happens to constitute part of an aggregate of which the other cells come from lower and less active stem levels, it is possible that the most active cell group may determine a new gradient,



FIGS. 45–52.—Apical sectors and apolar forms. Figs. 45–50, apical sectors, i.e., radially incomplete, apical partial forms, in polar aspect. Fig. 51, an apolar aggregate completely inclosed in perisarc. It underwent gradual decrease in size and secreted other layers of perisarc (not shown in figure) inside the first. After 10 days it had decreased to the size indicated by the inner circle, the space between the tissue surface and the outer perisarc being largely filled with perisarcal secretion in more or less definite layers. Fig. 52, a small apolar, naked aggregate showing ectoderm and entoderm separated by mesogloea.

of which it becomes the high end and therefore develops as an apical region and determines the development of other parts. A polarity determined in this way represents a new polarity but is dependent on the persistence from pre-existing individuals of physiological differences in different cell groups. Such differences are, of course, in-

dependent of the environmental factors directly affecting the aggregate; but if the differences were originally determined by environmental factors, the polarity determined by them is not independent of environment. Up to the present no evidence has been obtained that polarity is determined in this way in any of the aggregates. Moreover, if it were so determined, a much higher frequency of bipolar and multipolar forms than actually occurs might be expected, since many aggregates must undoubtedly contain more than one such active cell group and since each such group might become a new apical end. As a matter of fact, multipolar forms are much less frequent in aggregates than in large stem pieces. In the aggregates observed they constitute less than 2 per cent of the total; while in stem pieces, they often make up 30 per cent and may run as high as 50 per cent of the total (Child, 1926*b*, p. 781). Moreover, bipolarity has been found only in the apical forms, that is, the bipolar forms include only the distal manubrial regions or hypostomes, no bipolar complete hydranths or hydranths with stem having been found. All aggregates thus far observed that develop a complete hydranth, or the larger part of a hydranth, develop also a basal end (see Figs. 16, 17, 22–36).

If environment plays a part in the determination of polarity in the aggregates, the factor of differential exposure in relation to the substratum deserves consideration before other factors, since it has been found to be highly effective in determining polarity in stem pieces (Child, 1926*b*, 1927*a*, 1927*b*) and since it seems to be the chief differential to which the aggregates are exposed. At first glance it would seem that direct observation of the development of the aggregates should at once determine whether or not differential exposure plays any important part in determining polarity; for if it does play such a part, the polar axes should in general be vertical or nearly vertical to the substratum, though other factors might conceivably alter the direction in some cases. Direct observation, however, meets with certain difficulties. The aggregates do not adhere to the substratum until perisarc develops; and if the polar axis is vertical to the substratum, elongation in the direction of this axis is likely to make the original position of the aggregate unstable, particularly since slight movements occur and the mass is likely to tip

over and lie on one side without ability to right itself until the perisarc becomes attached. Successive observations at short intervals have shown that such change in position actually occurs in many cases, but in some aggregates the first distinguishable polar elongation takes place parallel to the surface of the substratum. It may be regarded as probable that in such cases the polar axis was originally vertical to the substratum and that the position of the aggregate was altered by slight movements before elongation became appreciable. In the absence of orienting landmarks, however, this remains only a probability. Localized marking of the aggregates has not yet been attempted. It was noted above that many of the larger aggregates give off cells from their lower surfaces. In such cases the cell débris usually serves to stick the mass to the substratum and hold it in a definite position. If the accumulation of the débris and the resulting bacteria do not lead to death of other parts of the aggregate, such cases afford favorable material for observation of the direction of the polar axis with reference to the substratum. In all such cases observed, the polar axis has been found to be approximately vertical to the substratum. In the short apical forms (Figs. 37–44) shortening instead of elongation in the direction of the polar axis usually occurs, and the lower pole of the aggregate often becomes flattened against the substratum and develops as the proximal region. As soon as flattening begins, the position becomes relative stable; and in those cases in which the apical end can be identified with certainty it apparently develops on the upper surface. But such aggregates, while still spherical, may change their positions to some extent in consequence of their contractile movements, so that even here there is uncertainty; moreover, it is often impossible to determine whether the apical forms are unipolar or bipolar. In short, direct observation, as far as it goes, indicates the significance of differential exposure in relation to the substratum as a factor in determining polarity; but it does not permit definite conclusions for all cases.

FORM FREQUENCIES IN RELATION TO DIFFERENTIAL EXPOSURE

With the earlier lots of aggregates, no special precautions were taken against changes of position in handling or in change of water,

the chief purposes of these experiments being to determine whether the aggregates would develop and to observe the course of development. Since these experiments made it probable that differential exposure was a factor in determining polarity, the attempt was made with later lots to determine the influence of this factor on polarity and form frequency. For this purpose the frequencies in undisturbed and in moved lots were determined; these are given in Table I. The table includes only the aggregates which developed visible polarity. The original numbers totaled more than a hundred in both undisturbed and moved lots; but some of these died in the moved lots, and more than half died in the undisturbed lots. Two aggregates 0.4–0.5 mm. in diameter remained spherical and secreted perisarc over the whole surface, and a few others about 0.1 mm. in diameter also failed to develop beyond the two-layered stage.

Table I includes all experiments on position except two series in which the death-rate was almost 100 per cent. In the table different lots—IA, IB, etc.—of the same series which developed under the same conditions are grouped together. All lots under the same series number, e.g., 53, represent material from one dissociation of fifty or more stems.

The column headings of the table require no explanation, but it may be noted that all apical partial forms are grouped together whether they are definitely unipolar, bipolar, or uncertain, radially complete, or sectors. In view of the uncertainty in so many cases, this seemed the only possible procedure. Since the bipolar forms are all apical partial forms, the table gives no data on bipolar frequencies; but as a matter of fact, those clearly distinguishable as bipolar were found only in the moved lots. The apical forms are much more frequent in the moved than in the undisturbed lots; and since some of the uncertain apical forms are doubtless bipolar, it is evident that the frequency of bipolar forms is considerably higher in the moved than in the undisturbed lots.

The differences between undisturbed and moved lots in the table are striking. The undisturbed lots show a total of 79 per cent, the moved lots a total of 9 per cent of unipolar forms with bases—a difference of 70 per cent. On the other hand, the moved lots show 67 per cent more apical partial forms than the undisturbed. Multi-

polar forms are few in both. Differences in frequency so great as these seem to leave no room for doubt concerning the effect of differential exposure. Continued contact with, or proximity to, the substratum of one region of the aggregate favors to a marked extent the development of basal ends. The forms which possess basal ends are either complete individuals (Figs. 22–30) or mosaic forms consisting of more or less of the distal hydranth region with a basal

TABLE I

FORM FREQUENCIES IN UNDISTURBED AND MOVED LOTS

Lots	Unipolar Forms with Bases	Apical Partial Forms: Unipolar, Bipolar, Sectors	Multipolar
Undisturbed			
53 IA, IB, IC	1	1	1 (Fig. 16)
59 IA, IB, IC, ID	4	7	0
61 I	5	0	0
62 IA, IB, III	27	1	0
Total	37	9	1
Percentage	79	19	2
Moved			
53 II	1	4	4 (Fig. 17)
59 IIA, IIB, IIC, IID, III	0	47	0
61 IIA, IIB	6	13	0
62 II	0	4	0
Total	7	68	4
Percentage	9	86	5

end (Figs. 31–36), that is, they represent either complete polar axes or the two terminal regions of such axes—in any case, more complete polar axes than the apical forms which represent only the extreme apical regions.

The point should be emphasized that in these aggregations the development of complete individuals and of apical forms is not merely a matter of size of the aggregate. As the figures show, the apical forms (Figs. 37–50) may be very much larger than many of the whole individuals (Figs. 25–30) or mosaic forms (Figs. 31–36). It

is true that the largest aggregates which have lived to develop (approximately 1 mm. in diameter in spherical form) have given rise to complete individuals, and the smallest aggregates, chiefly to apical partial forms; but within certain limits of size the result depends very largely on the conditions, that is, whether the aggregates are left undisturbed or undergo change in position. In the lots of Table I the aggregates which developed apical forms were often much larger than those which developed whole individuals.

Table II illustrates this independence of size in form frequency. Lot 1 of Table II consisted orginally of 20 aggregates 0.3–0.5 mm.

TABLE II

FORM FREQUENCIES IN TWO LOTS, BOTH OF WHICH WERE MOVED AND BROUGHT INTO FRESH SEA WATER ONCE DAILY DURING 4 DAYS AND THEN LEFT UNDISTURBED*

LOTS	NO. OF AGGREGATES	SIZE (MM.)	TIME OF DEVELOPMENT (DAYS)	UNIPOLAR FORMS WITH BASES		APICAL PARTIAL FORMS, UNIPOLAR, BIPOLAR, SECTORS	
				No.	Percentage	No.	Percentage
53 ID 1.....	18	0.3–0.5	4–5	1	5.6	17	94.4
53 ID 2.....	71	Less than 0.3	5½–7	46	65	25	35

* The aggregates of Lot 2, being smaller than those of Lot 1, developed more slowly than those of Lot 1; consequently their development of polarity occurred chiefly during the undisturbed period, and a high frequency of unipolar forms with bases resulted.

in diameter. These were moved about and turned over once daily during 4 days following aggregation. At this time 14 of them had developed far enough so that their character could be determined and recorded, and 2 had died. During the next 24 hours the remaining 4 developed, that is, all that lived had developed in 4–5 days, as the column headed "Time of Development" shows. Of these, only 1, or 5.6 per cent, developed with a base; and 17, or 94.4 per cent, developed as apical forms.

Lot 2 consisted of numerous smaller aggregates, less than 0.3 mm. in diameter. This lot was also moved and turned over once a day during the first 4 days following aggregation; but since the smaller aggregates develop more slowly than the larger, none of Lot 2 had developed even visible polarity within these 4 days, all

being still spherical. From the fourth day on, this lot remained undisturbed, and a day and a half later some had developed, and others developed up to the seventh day. In spite of the small size of the aggregates this lot showed 46 unipolar individuals with basal ends—that is, 65 per cent—and only 25 apical forms—35 per cent. Evidently the change in position during the first 4 days had little effect on the form frequency, because development did not progress far enough during that period for the effect to take place; but the absence of movement after 4 days occurred at the critical period and determined the development of basal ends on 65 per cent of the aggregates, although all of them were smaller than any of Lot 1. Some of the smallest individuals and mosaic forms found came from this lot.

Tables I and II show beyond any question that differential exposure of different parts of the aggregate lying on the bottom is an important factor in determining the character of the form which develops. But since the frequency of multipolar forms is very low, and since many of the apical forms which develop in the moved lots are unipolar, the question at once arises whether differential exposure is concerned in the determination of polarity, or merely in determination of development of a basal end and stem. The occurrence of bipolar forms in the moved lots, and not, so far as could be determined, in the undisturbed lots, seems to indicate that differential exposure does play some part in determining polarity. Moreover, further consideration shows that differential exposure determines the development, at least usually, of most of the length of the polar axis, since it determines complete individuals instead of apical partial forms; and it certainly determines development of the basal end, which sooner or later becomes a secondary gradient opposite in direction to the hydranth-stem gradient (Child, 1926*a;* Child and Hyman, 1926). These facts show that differential exposure is a very important factor in determining polarity. The moved lots develop much less polarity, that is, much less of the polar axis, than the undisturbed; in other words, the differential in a moved aggregate is, in general, much less than in the undisturbed, consequently structure develops on a larger scale. But the presence of even this slight differential in the moved lots requires consideration. There is no evi-

dence to indicate that it depends on the presence of cell groups in which differences of activity persist from the original stems. The facts indicate that it is determined by differential exposure; and since the changes in position are not continuous, but occur only at intervals of at least several hours, the undisturbed periods, particularly the overnight periods, provide adequate opportunity for differential exposure to become effective to some extent. Under such conditions a slight differential—that is, a partial gradient—is to be expected in most cases. Further experiment with more frequent or continuous change of position will be of interest.

VARIATIONS IN SIZE AND PROPORTIONS IN THE DEVELOPMENT OF AGGREGATES

The forms developing from aggregates show a very wide range of variation in size and proportions. The scale of organization of particular regions differs very greatly in different aggregates and is to a considerable degree independent of the size of the aggregate.

This great variation in scale of axiate organization constitutes further evidence that environmental conditions are concerned in the determination of polarity, for polarity is, of course, the fundamental factor in determining the scale of organization along the polar axis. Considering first the complete individuals—that is, those in which all parts along the polar axis are present (Figs. 16, 17, 22–30)—, it is clear from the figures that the length of the individual may range from that of Figures 16, 17 and 22 (2.5–3 mm.) to that of Figure 30 (0.35 mm.). This difference in scale is evidently associated with differences in size of aggregate, for in all cases the individual includes all of the aggregate. But marked differences in the relative proportions of parts along the polar axis appear. Figures 17, 24–26, 28–30) approach more or less closely the proportions of embryonic development, though some variations occur here, e.g., as regards relative size of hydranth and stem region. In some cases also the holdfasts develop much earlier than in others (Figs. 24, 26, 30). In other complete individuals, considerable departures from embryonic proportions occur. Forms like Figures 22 and 23, with relatively very large hydranths, scarcely a trace of stem, and a well-developed but short basal end, are frequent in a wide range of size. In such cases

the distal regions are, so to speak, laid out on a much larger scale than the proximal. In later development more or less approach to the usual proportions commonly occurs in consequence of differential growth, or of growth of the smaller at the expense of the larger parts; but this does not decrease the significance of the proportions in earlier stages. On the other hand, forms like Figure 27, with small hydranth and long perisarcal region, are also frequent. These extreme variations in proportion suggest the probability that the scales of organization of distal and proximal parts may be determined more or less independently of each other and that such independent determination is to be expected if differential exposure is concerned in determining polarity. But if the aggregates gave rise only to complete individuals, the assumption of an inherent organizing factor might be justified in spite of the variations, for these could be interpreted as effects of environment on the inherent factor. Consideration of the various types of partial forms, however, renders such an assumption highly improbable. The mosaic forms, for example, consist of distal and proximal regions without the middle regions of the polar axis. The usual types of mosaic forms observed are shown in Figures 31–36. Figure 16 is also a mosaic as regards the basal end on the manubrium; and mosaic forms of various sorts are of frequent occurrence in stem pieces, particularly under experimental conditions (Child, 1927*a*, Figs. 15–25). The common form of mosaic which develops from the aggregates consists of hypostome with distal tentacles and more or less of the manubrium and a basal region covered with perisarc. The region of the gonophores, the proximal tentacle region, and the naked stem are not represented. Such forms are more frequent among rather small aggregates (Figs. 33–36) but are not uncommon in aggregates much larger than many which give rise to complete individuals (Figs. 31, 32). Figure 32 shows the most extreme type of mosaic form in which the basal end is continuous with the distal manubrial region. As regards amount of material present, this aggregate was large enough to give rise to several complete individuals; but, like many others, it actually did develop an apical region on a very large scale and a small basal end. That an inherent organizing factor can be responsible for such forms is extremely improbable; but if polarity may be determined by dif-

ferential exposure, such forms are to be expected. None of the forms shown in Figures 31–36 ever developed any indications of proximal hydranth regions or naked stems. It is possible that if they could have been fed, the missing parts might have developed in the course of time, at least in some cases; but the fact remains that they were apparently not included in the original organization.

In the apical forms (Figs. 37–50) the same great difference in scale appears as in the other forms, and apparently without any relation to size of aggregate. The largest radially complete apical forms (Fig. 37) develop from aggregates large enough to form a large number of complete individuals, such as develop from small aggregates; and the largest apical sectors are almost as large (Fig. 45). Aggregates of any size below this, down to about 0.1 mm. spherical diameter, may give rise either to unipolar, radially complete apical forms or to sectors; and all except perhaps the smallest may give rise to bipolar apical forms (Figs. 18–21). Here, again, it is difficult to conceive of any inherent polarity or organizing factor which could determine so small a part of the whole on so wide a range of scale and quite independently of the size of the aggregate, but both the partial character and the differences in scale accord with expectation if polarity is determined by differential exposure.

The particular conditions concerned in determining the sectorial, as distinguished from the radially complete apical forms, are as yet unknown. These forms occur both in undisturbed and in moved lots. The radially complete apical forms are incomplete as regards polarity, and these sectorial forms are incomplete as regards symmetry. That their occurrence is not independent of environment seems evident, and that differential exposure after determination of the polar axis may play a part in determining the scale of radial organization seems probable; but further experiment is necessary for definite conclusions.

The few apolar forms which occur are more frequently small aggregates (Fig. 52), but occasionally an aggregate 0.5–0.6 mm. becomes inclosed in perisarc (Fig. 51). If such forms do not develop later and emerge from the perisarc, they may remain alive for 2 or 3 weeks or even longer, without appreciable change except gradual decrease in size and secretion of other layers of perisarc inside the first.

In general the aggregates, except those inclosed in perisarc, show slight contractility and change of shape in the spherical stage; and in the course of further development the characteristic motility of the parts present appears. The tentacles of the apical forms usually become extremely active after stimulation, e.g., the touch of a needle. In the apical sectors considerable changes of shape occur through muscular activity. For example, a sector like Figure 46 when at rest attains the form of Figure 47 by contraction of the circular muscles.

THE DEVELOPMENT OF TENTACLES

In general the distal tentacles develop before the proximal in the aggregates, as in embryonic development. This order differs from that usually found in reconstitution, in which the proximal tentacles usually develop first. In his study of embryonic development in *Corymorpha*, Torrey (1907) found that the tentacles do not appear in a definite order but that the sequence and relative positions of the first tentacles differ in different individuals. He believes, however, that a quartet arrangement is indicated for the distal, though not for the proximal, tentacles.

In the aggregates the appearance of the tentacles is, if anything, more variable than in embryonic development. The first three, four, or five distal tentacles may arise one after the other in various orders; or two, occasionally three, may arise almost simultaneously; but, so far as observed, slight differences in time appear always to exist. The first proximal tentacles often appear on the same side of the hydranth body as the first distal, but they may also appear elsewhere. It can scarcely be doubted that the localization of the first tentacles on one side of the body must be determined by some difference in physiological condition between this side and the other. In the aggregates, such a difference may conceivably result from slight physiological differences in the cells or cell masses constituting the aggregate, according to their origin from different regions of the stem. In view of the fact that the differences characteristic of different levels are rapidly lost in reconstitution, this suggestion seems scarcely adequate to account for all cases. Probably some slight environmental differential acting in a transverse direction is concerned in many, if not in all, cases. In certain cases the effective-

ness of such a differential can be directly observed, not only in the aggregates, but in the reconstitution of stem pieces. If the developing individual happens to lie on one side during a certain period of development, the tentacles appear first on the upper side (Child, 1926*b*). In this way slight differences in physiological condition are determined which give one side predominance as regards the start of tentacle development. Differentials of this sort may be slight and of relatively brief duration and may be altered in direction by changes in position of the developing form. In aggregates which are erect at the critical stage, various other environmental differentials may conceivably be concerned, e.g., light, water currents, etc. In the reconstitution of stem pieces, the tentacles develop in relation to the longitudinal entodermal canals, but in the aggregates there is no such pre-existing structure to localize development; moreover, the longitudinal canals do not develop in the hydranth.

Each developing tentacle unquestionably dominates more or less completely a certain area about itself, at least as long as it is actively growing; and this dominance prevents the development of other tentacles within the limits of that area. Outside that area and within the tentacle zone slight fortuitous differences in condition, depending directly or indirectly on environment, may localize other tentacles. The range of dominance of a developing tentacle may apparently differ widely. In the smaller aggregates, which develop more slowly than the larger, it apparently is less than in the larger. The more or less proportionate definitive size of tentacles and hydranth is probably a matter of relative ability of the various parts to obtain material for nutrition and growth. As in other forms, normal proportions are not fixed but may be altered by altering the gradient. Many of the apical forms never develop more than one or two distal tentacles; and in such cases these often become disproportionately long, even longer than the distal tentacles of hydranths developing on a much larger scale. Figure 40 is a case in point; Figure 43 another on a smaller scale. In such cases the single tentacle has apparently gained a predominance so great that it has inhibited entirely the development of others and in their absence has been able to obtain material for growth which they would have used had they developed.

The most important fact which appears at present from the observations on tentacle development in the aggregates is that the definitive radial symmetry is only gradually developed from an asymmetry which differs in different individuals. In many cases complete radial symmetry is never attained. That environmental factors are concerned in determining the initial asymmetry appears highly probable. If they are not concerned, either a different mechanism must be postulated in each individual or some sort of teleological controlling principle must be assumed.

DISCUSSION

Aggregation of cells from stem regions.—The rapid decrease in ability to aggregate during the first few hours following dissociation is undoubtedly connected with changes in the protoplasmic surfaces exposed to the medium. Apparently the exposed surfaces gradually develop a morphological membrane in consequence of the colloidal changes resulting from exposure to sea water. In fact, the gradual development of a highly refractive boundary of appreciable thickness is microscopically visible. A few preliminary experiments indicate that it will be possible to alter and control to some extent the capacity for aggregation. The gradual fusion of smaller rounded aggregates into approximately spherical, larger masses appears to be, at least in part, a surface-tension phenomenon, but may involve other factors, particularly in the larger masses. Its velocity is of the same order of magnitude as that of closure of cut ends and other wounds.

The high death-rate of the larger aggregates during the first 3 or 4 days following dissociation, particularly when they remain undisturbed, appears to result from a disproportion between surface and volume. They are solid masses of cells; and when they exceed a certain size, the respiratory exchange is probably inadequate for cells of the region adjoining the substratum and in the interior. If such aggregates remain in one position, that part of the surface in contact with the substratum is exposed to relatively low oxygen and high carbon dioxide, and disintegration often begins there before or at about the same time as in the interior. If the aggregate is able to develop to the two-layered stage with enteric cavity, the situation apparently becomes somewhat less acute; and if it survives un-

til elongation occurs, it usually continues to survive and develop. With decrease in size of the spherical stage, surface increases in proportion to volume, and the curvature of the surface is greater; consequently the region which closely adjoins the substratum is smaller. Obviously respiratory conditions in such aggregates are better than in the larger, and their susceptibility is much less than that of the larger. This interpretation of the greater viability of the smaller aggregates is, of course, hypothetical, but it seems to account satisfactorily for all the facts and other environmental factors which might determine such differences are not evident.

The behavior of dissociated hydranth cells.—Most of the dissociated hydranth cells behave very differently from those of the stem in showing no capacity for aggregation into solid masses. Since most of the hydranth cells may be regarded as more highly specialized than those of the stem, this difference in behavior is probably in some way associated with the higher degree of specialization. It is not clear, however, on what difference in the cells the difference in behavior depends. Certainly, naked protoplasm must be exposed following dissociation in the hydranth as well as in the stem, and it is evident from the anastomoses of protoplasmic strands from different groups of hydranth cells that union of protoplasm of different cells may occur. The cells do adhere to each other to some extent, but apparently because of slime rather than of protoplasmic continuity; and there is no approach to spherical form except in the few cell groups which behave like those of the stem. The origin of the few small aggregates of these cells within the hydranth is at present unknown. The only parts of well-developed hydranths, as were all those used for dissociation, in which active growth and development continue and which can therefore be regarded as in some degree embryonic, or at least less highly specialized than other parts, are the buds and early developmental stages of the medusoid gonophores. Development of new gonophores continues, even in advanced stages of the hydranths. That the capacity for aggregation is more likely to exist in these supposedly less highly specialized parts than elsewhere in the hydranth seems probable. Observation of the results of dissociation of these regions in the absence of other parts should permit a definite conclusion.

The origin of ectoderm and entoderm.—The question of the origin of the ectoderm and entoderm in the aggregates is of considerable interest. As already noted, histological study of the aggregates has not yet been made; and examination of the living material, while highly suggestive, has not led to positive conclusions. The following possibilities of origin may be noted: first, the entoderm cells taking part in aggregation may migrate to the interior and ectoderm cells to the exterior; second, entoderm cells in the interior and ectoderm cells on the exterior may grow and proliferate at the expense of cells which are out of their usual environment, and therefore undergo atrophy and resorption; third, ectoderm or entoderm cells or both may undergo dedifferentiation and redifferentiation. As is evident from the work of Torrey (1907, 1910), there are no cells in *Corymorpha* which correspond to the interstitial cells of *Hydra* and so might be regarded as indifferent cells capable of replacing others.

So far as observed, all the aggregated balls, including those only 0.1 mm. in diameter, or even somewhat less, develop ectoderm and entoderm in essentially normal relations and amounts. It appears highly improbable that each aggregate consists of the proper volumes of the original ectoderm and entoderm to provide for the formation of the two layers in normal amounts. Undoubtedly, some of the aggregates must consist in large part, perhaps wholly, of cells of one layer. It seems certain, therefore, that mere migration of ectoderm and entoderm cells into the proper positions cannot alone account for the development of the layers as they are found.

As regards the second possibility, viz., proliferation and resorption, we should expect, if it were an important factor in the development of the layers, that in some cases one or the other of the layers would be at first "too small" or "too large" and would undergo change in volume. Moreover, this method of origin, like the preceding, would completely exclude the possibility that an aggregate consisting wholly of cells of one layer could develop the other layer. As a matter of fact, no aggregates have as yet been found to remain permanently in the one-layered condition.

In short, the evidence at hand seems to indicate very clearly, though it does not prove, the occurrence of dedifferentiation and redifferentiation of either ectoderm or entoderm or both. In the

light of Torrey's work (Torrey, 1910) there appears to be no question concerning the ability of the ectoderm cells of the stem to develop into other ectodermal parts of the body than those which they originally represented. Torrey also describes regressive transformation of the axial parenchymal cells and their development into entodermal epithelium (Torrey, 1910, p. 207, Fig. 3). H. V. Wilson (1911) regards the development of new individuals from dissociated cells of hydroids as dependent on the dedifferentiation and redifferentiation of cells, and Braem (1908) showed that the buds of certain medusae develop from ectoderm alone and that entoderm arises from some of the cells which were originally ectodermal. In view of all these facts the transformation of ectoderm into entoderm, or vice versa, must at present be regarded as highly probable.

The two-layered spherical form.—This form, which results from the appearance of ectoderm and entoderm as definite layers in normal relation, apparently does not differ in any essential way from a blastula, but shows a much wider range of variation in size both above and below that of the blastula which develops from the egg. Apparently, also, it may represent purely a surface-interior pattern, with complete spherical symmetry; and if it does not develop some other differential, it may remain only that indefinitely. Under the conditions thus far employed, the very small aggregates, less than 0.1 mm. in diameter, usually, if not always, retain the spherical form indefinitely and develop no visible indications of polarity. In fact, it seems to be true that the smaller the aggregates, below a certain limit, the less frequently they develop polarity. The great majority of the aggregates above 0.1 mm. in diameter do not remain spherical indefinitely but develop polarity sooner or later, even when moved about periodically. The aggregates which secrete perisarc over the whole surface usually remain spherical indefinitely but may finally develop polarity and emerge from the perisarc, much as the planula emerges from the egg membrane (Fig. 15). The secretion of perisarc in any case indicates a change in physiological condition, for the perisarcal stem region was not included in the experimental material in any experiment. In the light of the fact that the apical regions of various hydroids may be transformed into perisarc-secreting stolons by depressing or inhibiting conditions (Child, 1923), and since

the perisarcal region of the stem represents the lower levels of the primary gradient, it appears probable that secretion of perisarc over the whole surface of an aggregate results from a depression or occurs in aggregates which, for some reason, are less active than others.

The polarity of the aggregates.—It is evident from the experiments that the cells of the naked stem regions of *Corymorpha*, like those of the stems of various other hydroids (Wilson, 1911), are able to aggregate and develop into new individuals or partial individuals. The structure of *Corymorpha* is more complex than that of the other hydroids used for such experiments; but the facts show that persistence of this structure, or even of the physiological conditions of which it is the expression, is unnecessary for the reconstitution of an individual. The problem of the origin of the new order or pattern in the aggregates has not been considered in the earlier studies of aggregation of dissociated cells but is of fundamental importance. It is the problem of the origin of polarity and symmetry in the forms developing from aggregates, and involves the question whether individual pattern is inherent in the species-protoplasm and independent of environment or whether it originates in relation and reaction of the protoplasm to environment.

The first indications of polarity usually consist in the gradual change of the spherical aggregate into the definite form of an individual or an apical partial form. In the development of complete individuals the first visible change from the spherical form is elongation in the direction of the polar axis. The basal region may begin to secrete perisarc while hydranth and stem are still in a very early stage (Figs. 29, 30, 34, 36). In the hydranth-stem region development progresses on the whole from the apical end basipetally, distal tentacles developing before proximal, as the figures show. This is similar to the course of embryonic development and different from that in most cases of reconstitution, in that there proximal tentacles develop before distal. In reconstitution the region from which hypostome and manubrium develop apparently must undergo more extensive physiological change before it can develop these more distal regions than the region which becomes the proximal part of the hydranth. On the basis of the gradient conception this is to be expected, for the distal region of the hydranth represents a higher level

of the gradient, that is, a greater change from the physiological condition of the stem. In the aggregate, however, the pre-existing stem organization has been obliterated, so that the aggregate resembles much more nearly the embryo than it does the reconstituting stem piece, as regards the course of development along the axis. The aggregate differs widely from the embryo, however, in that under the experimental conditions employed, it may give rise to a single complete individual, a multipolar, or a partial form; and the partial form may be either a mosaic, consisting of the two terminal regions of the axis, or a bipolar or unipolar apical region, or an apical sector.

No indication of a gradient has been found in any case in the earlier stages of the spherical aggregates; but when elongation begins, the hydranth-stem gradient becomes distinct and, the secondary gradient in the opposite direction (Child, 1926*a;* Child and Hyman, 1926) becomes distinguishable sooner or later. Attention was called above to the possibility that in at least some cases a cell group from near the distal end of the stem might aggregate with other cells from lower stem levels and so become the high end of a new polar gradient and develop into the apical region. Thus far there is no evidence to indicate that anything of this sort occurs. If it were of common occurrence, the frequency of multipolar and and bipolar forms should be much higher than it is.

The high frequency of unipolar forms indicates either the action of some definite external differential or an inherent polarity. But the development of apical partial forms from large as well as from small aggregates, and of complete individuals from small as well as large aggregates, and of mosaic forms from aggregates of all sizes is difficult to reconcile with the conception of an inherent polarity. If such a polarity existed, all aggregates, except perhaps the smallest, should give rise to complete individuals. Actually, however, very different forms develop from aggregates of the same size, and similar forms from aggregates of different size. The scale of the axiate pattern may differ very greatly in different cases and quite independently of the size of the aggregate. An aggregate may give rise to an apical end only (Fig. 37), though many times as large as another which develops into a whole individual (Fig. 30) or a mosaic (Fig. 36). Again, an aggregate may develop a very large hydranth and

small base (Fig. 22); others of the same size or smaller may give rise to absolutely or relatively smaller hydranth and larger base (Figs. 16, 17, 27). In short, under the conditions of experiment the scale of organization and of proportions of different parts may differ very widely without relation to size of aggregate. If we attempt to interpret these differences in terms of an inherent polar organization, it will be necessary to assume an organization of different character for each of them; and the high degree of uniformity in embryonic development offers little basis for such assumptions. It seems to be evident that we cannot satisfactorily account in terms of an inherent polarity for the development of the aggregates as it occurs.

On the other hand, the frequencies of occurrence of the various forms may be controlled and altered to a high degree by controlling the environmental differential of position, that is, the differential exposure of the aggregate with respect to the substratum. Thus far the control has consisted merely in allowing some lots to remain undisturbed and changing periodically the positions of others. It was found that the frequency of forms with basal ends was about 70 per cent higher in the undisturbed than in the moved lots; and that of apical partial forms, almost 70 per cent higher in the moved than in the undisturbed (Table I). Evidently the periodic change of position favors axial development on a larger scale of pattern than maintenance of the same position, so that the moved aggregates tend to develop as apical regions only, instead of complete individuals or mosaics with basal ends. Table II showed further that even after several days of periodic change of position a comparatively short undisturbed period was sufficient to increase by some 60 per cent the frequency of unipolar forms with basal ends.

All the data agree in showing that the greater and more continuous environmental differential to which the undisturbed aggregates are exposed determines a high frequency of complete individuals and mosaics with basal ends; while periodic change in position, and so in direction of the environmental differential, determines a high frequency of apical partial forms, some of which are bipolar. In terms of physiological gradients, these differences in frequency mean that in the aggregates which remain undisturbed the differ-

ential is sufficient to determine the development of the whole axial gradient in a high percentage of the cases; while in those moved about, the differential is less marked and only the apical portion of the polar gradient appears. In the larger aggregates, this portion of the gradient may be on a much larger scale than in the undisturbed lots. In other aggregates, two partial gradients are determined in different or opposite directions, giving rise to bipolar forms. And in still others, the distal and the basal portions of the polar gradients are determined on so large a scale in relation to size of aggregate that intervening regions are not represented and a mosaic form results. In short, all the modifications of axial development may be readily and simply interpreted in terms of gradients determined by differential exposure. Certain aspects of the data, however, require some further consideration in the light of such interpretation.

The fact that even the apical partial forms which develop from the moved lots are frequently unipolar may seem at first glance to conflict with the interpretation of polarity as a gradient determined by differential exposure. As a matter of fact, however, even the moved lots were moved only at intervals of at least several hours, and the undisturbed periods were often long enough to initiate a gradient. The repeated movement and change of position provided more favorable conditions than in the undisturbed lots, consequently the gradient in such aggregates was likely to be on a large scale, so that even an aggregate of considerable size might develop only the apical region.

Another fact to be accounted for is that thus far no cases of complete or almost complete hydranths without stems or basal regions have been found among the aggregates, though such forms are of frequent occurrence in short stem pieces (Child, 1926*c*). The aggregates either have given rise to the extreme apical regions only or have developed a basal end as a mosaic or a complete individual. Experiments with undisturbed and moved lots of stem fragments 1 mm. or less in diameter, obtained by chopping stems with a scalpel, show that hydranths without stems may or may not appear, according to experimental procedure, and indicate that their absence in the aggregates is incidental and the result of the experi-

mental conditions. Probably with more frequent change of position many of the mosaic forms (Figs. 31–36) would have been hydranths without stems or bases.

Another striking fact is the low frequency of multipolar forms in the aggregates as compared with stem pieces (Child, 1926*c*). In the great majority of multipolar forms arising from stem pieces the two cut ends give rise to hydranths; and the region between, or often that part of a cut end which adjoins the substratum, develops one or more basal ends. Occasionally more than one hydranth or manubrium develops from one cut surface. In the aggregates there are no cut surfaces to serve as factors determining development of more than one polarity. Moreover, simultaneous determination of two or more gradients never occurs in the aggregates under the conditions thus far employed. One gradient must always be determined by one position before another is determined by another position. This being the case, a relatively low frequency of bipolar and multipolar forms is to be expected, but their frequencies could undoubtedly be altered by more frequent change of position.

And finally, the great differences in proportion of distal and proximal parts in the same form are inexplicable in terms of an inherent polarity but are readily accounted for in terms of gradients. For example, a moved aggregate developing as an apical end on a large scale may rest in one position long enough to determine a basal end on the region adjoining the substratum (Fig. 32). Again, the upper surface of a large aggregate may be active enough, because of its distance from the bottom, to determine hydranth and stem on so large a scale that the basal end is relatively small (Fig. 22). On the other hand, the smaller undisturbed pieces are likely to be more or less inhibited because of the nearness of all parts to the bottom and so develop shorter apical gradients which determine relatively smaller hydranths and larger basal ends (Figs. 24–27).

Tentacle development.—Incidental observations on the development of the tentacles of aggregates show that the definitive radial arrangement of the tentacles develops out of a radial asymmetry comparable to a dorsoventrality and that there is no uniformity in different individuals. The position of the first tentacle or tentacles has not been shown to be associated with any particular environ-

mental differential; but it seems evident that the developmental precedence of a particular radius of the tentacle zone in the aggregates, as in embryonic development (Torrey, 1907), must result from some difference in exposure to environmental factors. Such differential exposure may be purely fortuitous, but it is evident that something must have happened to determine slight quantitative differences in different radii. When a tentacle is once localized and begins to develop, it apparently dominates the tentacle zone within a certain distance and prevents the development of other tentacles within that distance. Consequently the next tentacles to develop usually arise as far from the first as the size of the developing manubrium permits, or at least at a considerable distance from it. In later stages, as the growth-rate of the tentacles decreases, their range of dominance apparently decreases and other tentacles appear between those already present. In this way the final radial arrangement of tentacles gradually develops out of the radial asymmetry, and the course of development is different in different individuals. But, given a certain range of dominance, varying with physiological condition, in each tentacle of the tentacle zone, the final radial pattern is easily accounted for—in fact, appears to be a necessary result. That the tentacles do mutually influence each other is further suggested by the growth of distal tentacles to an unusual length when only one is present (Figs. 40, 43).

Apical sectors.—The particular conditions determining the forms called apical sectors (Figs. 45–50) are as yet unknown, but the radial asymmetry of these forms indicates very clearly the action of an environmental differential which determines the precedence of one side of the apical region and its development on so large a scale that the aggregate is not large enough to develop the whole circumference. In terms of reaction to environment, these radially incomplete forms are as readily accounted for as are forms incomplete in the direction of the polar axis. Moreover, if symmetry is not fundamentally different from polarity, incomplete symmetry is to be expected as well as incomplete polarity. The few observations already made on the development of these sectors indicate that they arise through extreme precedence of one side. It is certain that they are not the result of sudden rupture along some meridian of radially

complete forms. In these forms the relation of ectoderm and entoderm is different from that in other forms, for the entoderm is not internal to the ectoderm but constitutes one side of the form. The question how this relation is determined is of considerable interest but requires further investigation. In the earliest stages in which the sectors are recognizable as such, the asymmetry is apparently present. In any case, the sectors appear to represent a radial asymmetry of still greater degree than that which appears in tentacle development. In fact, the asymmetry is so great in these forms that the limited size of the aggregate renders impossible the attainment of radial symmetry.

To sum up: The data on the development of polarity in the aggregates can be satisfactorily interpreted in terms of a primarily quantitative physiological differential—that is, a gradient, originating in relation to an environmental differential—and at present it appears impossible to account for them in other terms. Together with the other experimental data on polarity in *Corymorpha* (Child, 1926*a*, 1926*b*, 1927*a*, 1927*b*, 1927*c*, 1928; Child and Hyman, 1926), they constitute a body of evidence which is at least highly significant in its bearing on the problem of physiological polarity. Moreover, the radial symmetry of the hydranth is evidently not inherent in the protoplasm but develops gradually from a radial asymmetry which is apparently the expression of slight, fortuitous physiological differences on different sides or in different radii of the body. In the sectorial forms, this asymmetry is so great that the attainment of radial symmetry becomes impossible because of the limited size of the aggregate. In general, the development of the aggregates indicates very clearly that the *Corymorpha* individual represents not merely the specific *Corymorpha* protoplasm but rather the reactions of this protoplasm to environmental differentials and the records in the protoplasm of such reactions.

SUMMARY

1. Cells and cell groups of the naked stem region of *Corymorpha palma*, dissociated by grinding with sand until most of the fragments are 30–40 μ in diameter and then strained through bolting-cloth, are capable of aggregation during the first few hours following dissociation.

2. Aggregation apparently results from chance contacts, not from directed movement.

3. The ability to aggregate decreases from the time of dissociation and under the conditions of experiment—temperature 22° C., standing sea water, diffuse daylight—and disappears within 5–6 hours, apparently in consequence of development of precipitation membranes on the exposed surfaces.

4. When the dissociated material is allowed to settle in tubes, aggregation occurs during the settling and on the bottom. The aggregates are at first irregular flattened masses or sheets, but gradually they approach or attain spherical form.

5. Spherical aggregates above 1 mm. in diameter show a very high death-rate during the first 2–3 days following dissociation, apparently because of the disproportion between surface and volume. Among the smaller aggregates the death-rate is usually low.

6. When the aggregates first become spherical, they are solid masses of cells in which it has not yet been possible to distinguish ectoderm and entoderm. The large entodermal parenchymal cells are destroyed by the process of dissociation.

7. Within 2–4 days ectoderm and entoderm become distinguishable in normal relation and amounts. The facts at hand indicate, but do not prove, that dedifferentiation and redifferentiation of ectoderm or entoderm or both occur.

8. The spherical, two-layered aggregates resemble blastulae but may range far above and below normal blastulae in size.

9. In later development those aggregates which give rise to complete individuals elongate and resemble planulae, and the development of the hydroid takes place essentially as in ontogeny. In the development of partial forms, the elongation does not necessarily occur.

10. Most hydranth cells after dissociation do not aggregate as do the stem cells, though they may cohere slightly, probably on account of slime secretion. The cells or cell masses show more or less amoeboid change of shape and may migrate and anastomose or separate. Single cells showing progressive amoeboid movement are found among dissociated hydranth cells.

11. Occasional small aggregates occur in hydranth dissociations.

It is suggested that these may consist of cells from the regions of gonophore buds, this being presumably the least highly specialized region of the hydranth.

12. The aggregates of dissociated stem cells may develop into unipolar, bipolar, or multipolar forms; but under the conditions thus far employed, unipolar forms are more frequent than others.

13. The unipolar forms may be complete individuals, or so-called "mosaic" forms, consisting of adjoining apical and basal regions without parts normally intervening, or may be apical partial forms, consisting of the distal part of the manubrium or the hypostome, with or without distal tentacles, or may be sectors of the apical region.

14. The scale on which development takes place, and therefore the kind of form which develops from an aggregate, is to a high degree independent of size of aggregate. Apical partial forms may develop from aggregates many times the size of others which give rise to complete individuals.

15. Apical and basal regions of the same polar axis may develop on very different scales, so that great differences in proportion of these parts appear independently of size of aggregate.

16. Development of aggregates in contact with the substratum without change of position gives a high frequency of unipolar forms with base (79 per cent), either complete individuals or mosaic forms. Development with change of position once to three times a day gives a high frequency of apical partial forms (86 per cent).

17. The data show that frequent change of position determines development of the polar axis on a larger scale than in the aggregates which remain undisturbed.

18. All the evidence from the experiments indicates that the polarity of the aggregates is determined by the environmental differential between upper free surface and surface in contact with the substratum, and that polarity is primarily a quantitative differential in physiological condition.

19. The radial symmetry of tentacle arrangement in the mature hydranths develops gradually from a radial asymmetry, and the course of its development differs in different individuals. The localization of the first tentacle or tentacles is apparently determined

by some slight differential in a certain radius or certain radii of the tentacle zone. If this is the case, and if each developing tentacle has a certain range of dominance varying with physiological condition, the definitive radial arrangement is readily accounted for.

20. The apical sectors apparently represent asymmetric radial development on so large a scale that the size of the aggregate permits only a sector to be formed. Such forms are partial with respect to the radial pattern, just as the other apical partial forms are partial with respect to polar pattern.

LITERATURE CITED

BRAEM, F. 1908. Die Knospung der Margeliden, ein Bindeglied zwischen geschlechtlicher und ungeschlechtlicher Fortpflanzung. Biol. Zentralbl., XXVIII., 790.

CHILD, C. M. 1923. The axial gradients in Hydrozoa. V. Biol. Bull., XLV, 181.

———. 1926*a*. Studies on the axial gradients in *Corymorpha palma*. II. Biol. Gen., II, 609.

———. 1926*b*. Studies on the axial gradients in *Corymorpha palma*. III. *Ibid.*, II, 771.

———. 1927*a*. Modification of polarity and symmetry in *Corymorpha palma* by means of inhibiting conditions and differential exposure. I. Jour. Exp. Zoöl., XLVII, 343.

———. 1927*b*. Modification of polarity and symmetry, etc. II. *Ibid.*, XLVIII, 197.

———. 1927*c*. Experimental localization of new axes in *Corymorpha* without obliteration of the original polarity. Biol Bull., LIII, 469.

———. 1928. Experimental transformations of bipolar forms in *Corymorpha palma*. *Ibid.*, LIV, 1.

CHILD, C. M. and HYMAN, L. H. 1926. Studies on the axial gradients in *Corymorpha palma*. I. Biol. Gen., II, 355.

FÖYN, B. 1927. Studien über Geschlecht und Geschlechtszellen bei Hydroiden. II. Arch, Entwickelungsmech., CX, 89.

GALTSOFF, P. S. 1925. Regeneration after dissociation (an experimental study on sponges). I. Behavior of dissociated cells of *Microciona prolifera*, Verr. Jour. Exp. Zoöl., XLII, 183.

HUXLEY, J. S. 1921. Further studies on restitution bodies and free tissue culture in *Sycon*. Quart. Jour. Micr. Sci., LXV, 293.

MÜLLER, K. 1911. Das Regenerationsvermögen der Süsswasserschwämme, insbesondere Untersuchungen über die bei ihnen vorkommenden Regenertion nach Dissociation und Reunition. Arch. Entwickelungsmech., XXXII, 397.

TORREY, H. B. 1907. Biological studies on *Corymorpha*. II. Univ. of Calif. Publ. Zoöl., III, 253.

———. 1910. Biological Studies on *Corymorpha*. III. *Ibid.*, VI, 205.

WILSON, H. V. 1907. On some phenomena of coalescence and regeneration in sponges. Jour. Exp. Zoöl., V, 245.

———. 1910*a*. Development of sponges from tissue cells outside the body of the parent. Bull. Bur. Fish., XXVIII, 1267.

———. 1910*b*. Development of sponges from dissociated tissue cells. *Ibid.*, XXX, 1.

———. 1911. On the behavior of the dissociated cells in hydroids, Alcyonaria and *Asterias*. Jour. Exp. Zoöl., XI, 281.